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13. SUPPLEMENTARY NOTES

14. ABSTRACT (around 200 words) Perfluorocarbon emulsions (PFCs) may use to treat traumatic injuries by enhanced delivery of oxygen. A concerned side effect of PFC may cause thrombocytopenia (TCYP). FDA requests investigation of the phenomenon to exclude Platelet (Plt) inflammatory/embolic safety risks prior to clinical trial. The results (phase I, n=32, 4 groups) showed that PFC given to the normal sheep did not significantly change the Plt number and activation compared with the control groups. In the Phase II & III studies, PFCs as an adjuvant resuscitation fluid were added to the hemorrhagic shock (n=39, 4 groups) or polytrauma (blast traumatic injury and hemorrhage, n=20, 2 groups) sheep after initial resuscitation with hespan until the mean arterial pressure reached to 55 mmHg. The results showed that the Plt number were reduced after immediately resuscitation in all injured groups. However, PFCs did not exaggerate the change of Plt number and activation comparing with non-PFC controls over the 7 survival days. Plt mean volume, Plt aggregation, Thrombin potential by CAT, Plt CD-62, fibrinogen level also showed no significant change compared with control groups. Quantitative Plt morphological activation (observation with scanning electron microscopy) was correspond with the results of functional assays. There are no significant percentage changes in circulating neutrophils and monocytes after PFC infusion in normal or injured sheep models. Therefore, the intravenous infusion of PFCs in healthy and hemorrhagic sheep would not cause massive or severe coagulopathy.

15. SUBJECT TERMS
Platelets, Platelet Activation, Perfluorocarbon emulsions (PFCs), Coagulation factors, Blast Injury, Hemorrhagic Shock, Resuscitation, Sheep

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1. INTRODUCTION

Perfluorocarbon emulsions (PFCs) are a small volume robust (temperature stable, long storage life, portable) intravenous (i.v.) fluid, easily carried by medics/corpsmen to site of first contact. PFCs enhance O2 solubility/diffusion from circulating red cells. PFCs have shown efficacy in animal models of hemorrhagic shock, tissue ischemia, decompression sickness (DCS), traumatic brain injury (TBI) and other important military applications. Our work and that of others demonstrated that PFCs enhance O2 delivery at normal FiO2 and that perhaps the most important aspect of PFC infusion was an enhanced O2 delivery from native erythrocytes to tissues. Furthermore, it appears that PFCs enhance O2 diffusion, thereby decreasing the barrier to non-polar gas movement made up of aqueous materials (plasma and extracellular fluids). However, a class-based side effect of PFC (day 2-5 after infusion in 30-50%) is thrombocytopenia (TCYP). The mechanism is inadequately investigated but is caused by reduced production or enhanced clearance (partial activation) of platelets (Plts). These safety concerns posed by the United States Food and Drug Administration (FDA) have to do with a potential risk of hemorrhage/thrombosis and inflammation related to PFC infusion. Casualty care for hemorrhage, gas embolism (blast and DCS) and TBI all involve degrees of inflammatory up-regulation and variable elements of coagulopathy. The current approved work is to answer safety and mechanism questions regarding causes/extent of thrombocytopenia after PFC infusion. Pertinent large animal models of normal and casualty scenarios will be investigated, thereby demonstrating whether the use of PFC in hemorrhage and blast TBI possess any added coagulopathic risk to future victims, compared to normal. Large animal models will examine specific causal hypotheses for TCYP and whether this exists as a class effect. In the end, the work will provide answers to questions blocking further development of PFCs. In this proposed study, the side effects of two PFC's on platelet count, structure and function will be tested. In the present study, the specific aims are to answer the following: #1 Whether PFC infusion significantly changes Plt number or activates Plts than the controls in vivo, #2 Whether Plt/white cells clumps (microaggregates) occur, and #3 Evaluate the mechanisms of partial Plt activation (if it occurs).

2. KEY WORDS:

Platelet number, Platelet Activation, Perfluorocarbon emulsions (PFCs), Coagulation factors, Hemorrhagic Shock, Blast Injury, Resuscitation, Sheep

3. ACCOMPLISHMENTS:

What were the major goals of the projects?

To test the main hypothesis, the following major tasks were included in the study:

Task1: Effect of intravenous infusion of PFCs (two different types of PFCs) on platelet number and activation as well as coagulation profile in the normal sheep.

Task2: Effect of intravenous infusion of PFCs (two different types of PFCs) on platelet number and activation as well as coagulation profile in the hemorrhagic shock sheep.

Task3: Effect of intravenous infusion of PFCs (two different types of PFCs) on platelet number and activation as well as coagulation profile in polytrauma sheep (blast trauma followed with hemorrhagic shock model).

What was accomplished under these goals?

Task1: Using normal sheep (ovine, 20-30 kg) model to test the effect of PFC intravenous infusion on platelet number and activation. Sheep were randomly divided into 4 groups

(Oxygent, Perftoran, hetastarch and saline/naïve groups, n=8/each group). Venous blood samples were collected at baseline, 0 minute after PFC infusion, 3, 24, 96 hours and 7 days post PFC infusion for Plt/white cell activation (Plt number, Plt white cell aggregates, flow cytometry-glycoprotein expression) and other coagulation data (RoTEM, Platelet Shear Modulus, PFA-100 and Plt aggregometry) Samples were also examined with scanning electron microscopy for Plt activation morphology. Specific objectives included: a) development of survival animal model following the IACUC/USDA guide line; b) measurement of platelet number and activation; c) coagulation factors (see appendix table1: list of measured parameters and table 2: summary results); d) exam the status of platelet activation with scanning electron microscope after PFC infusion comparing with hespan infusion or naïve control sheep).

Two types of PFC were used in the study: **Oxygent** (PFC 60% w/v, 5 ml/kg or 3 grams/kg, iv) and **Perftoran** (PFC 20% w/v, 5 ml/kg or 1 gram/kg, iv). Control groups included **Hespan** (6% Hetastarch, 5 ml/kg, iv) and **naïve** groups.

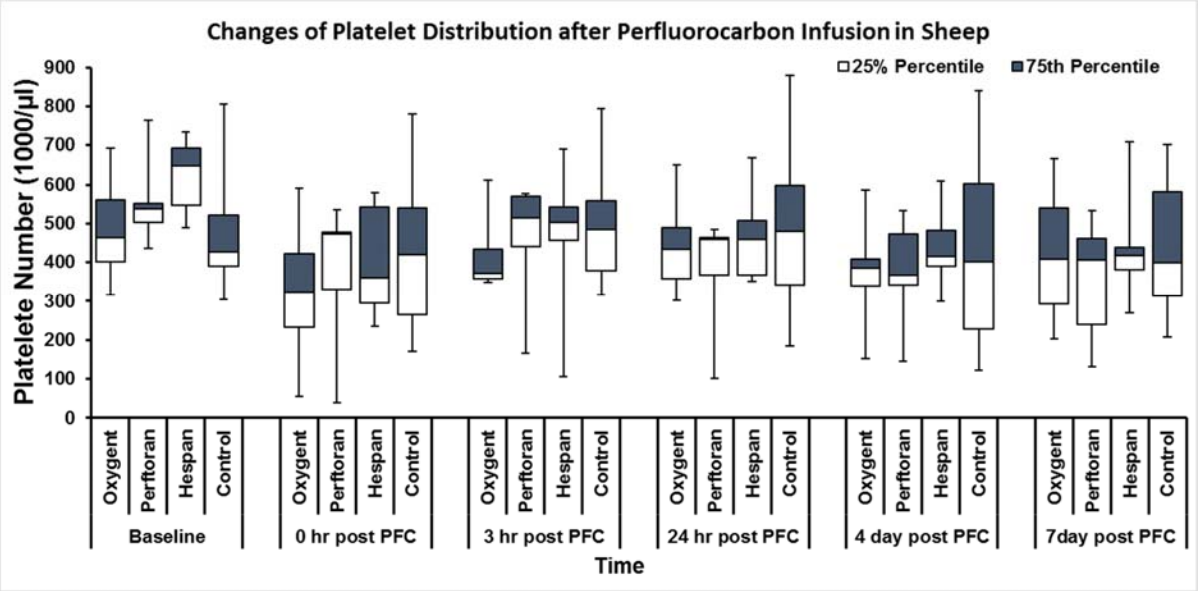


Figure 1 showed the changes of platelet numbers after PFC intravenous infusion compared with hespan infusion and naïve controls. Platelet number was reduced in all PFC and hespan infusion animals compared with their baseline and naïve group (median ± SD). The normal sheep platelet number is from 100,000 ~ 800,000 / microliter (mcL).

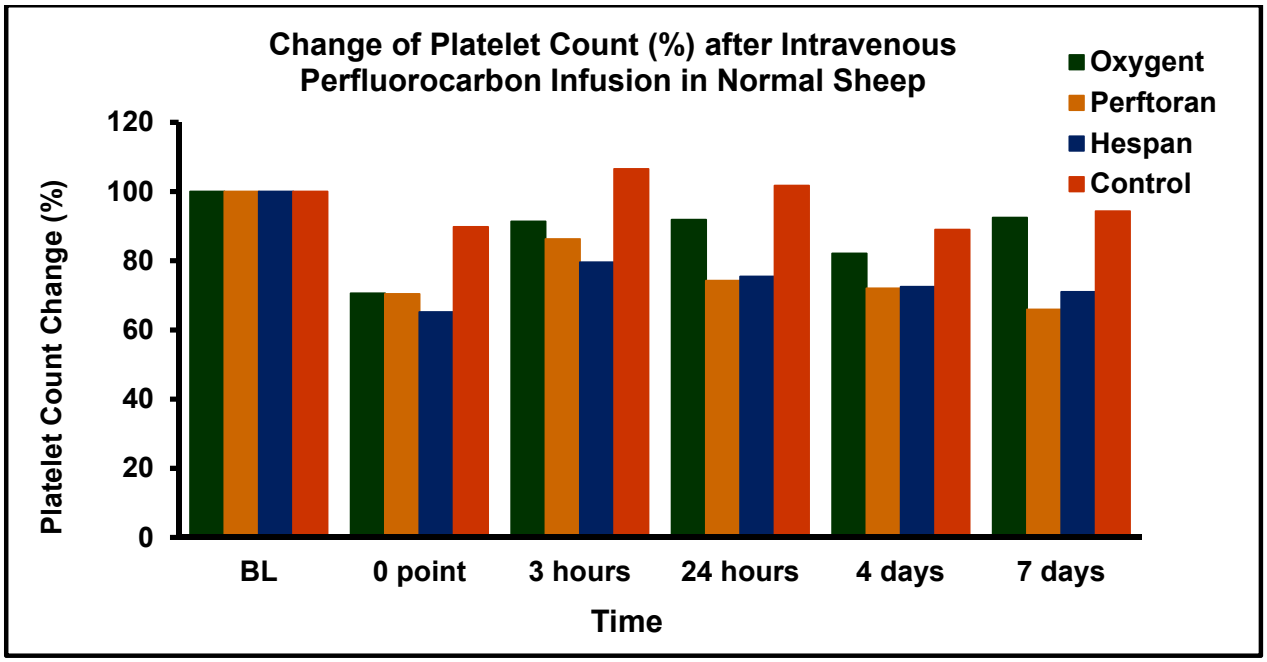


Figure 2 showed the percentage changes of platelet numbers after PFC intravenous infusion compared with hespan infusion and naïve controls. Platelet number was reduced right after the infusion (30% reduction at 0 point) in all PFC and hespan infusion animals compared with their baseline and naïve group. The percentage changes of platelet number in Oxgent group were less than 20% at all sample points except 0 point.

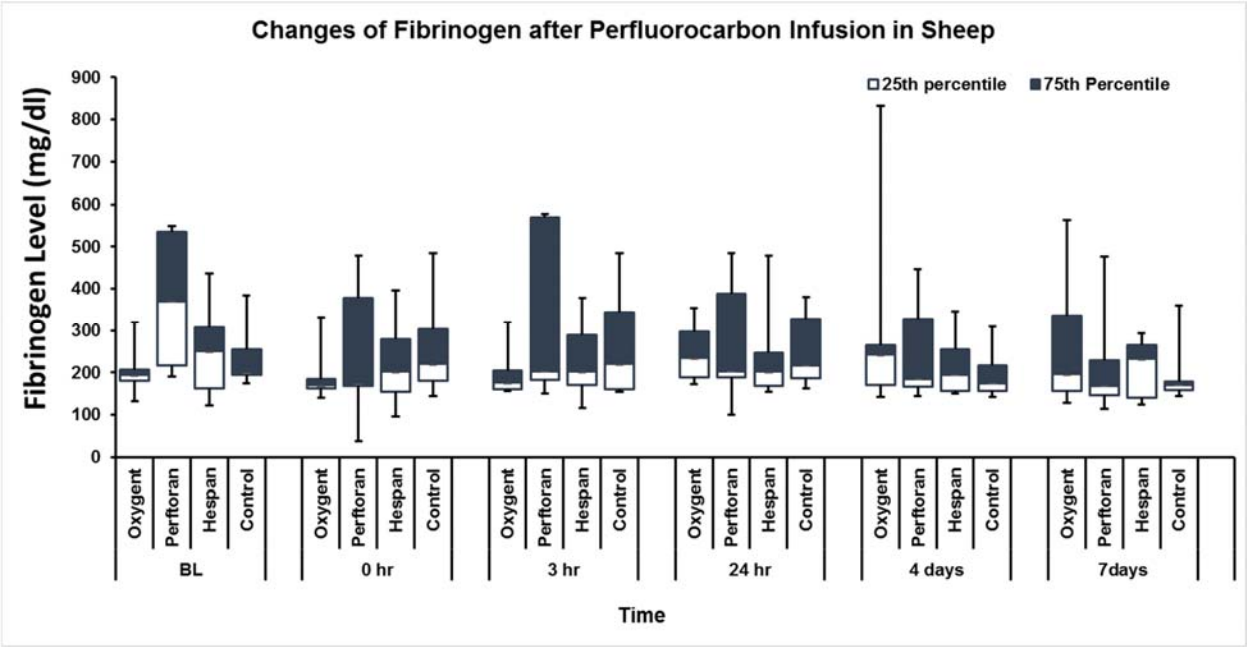


Figure 3 showed the changes of fibrinogen level after PFC intravenous infusion compared with hespan infusion and naïve controls. Fibrinogen level in PFC groups did not show a significant change compared with their baseline and naïve group.

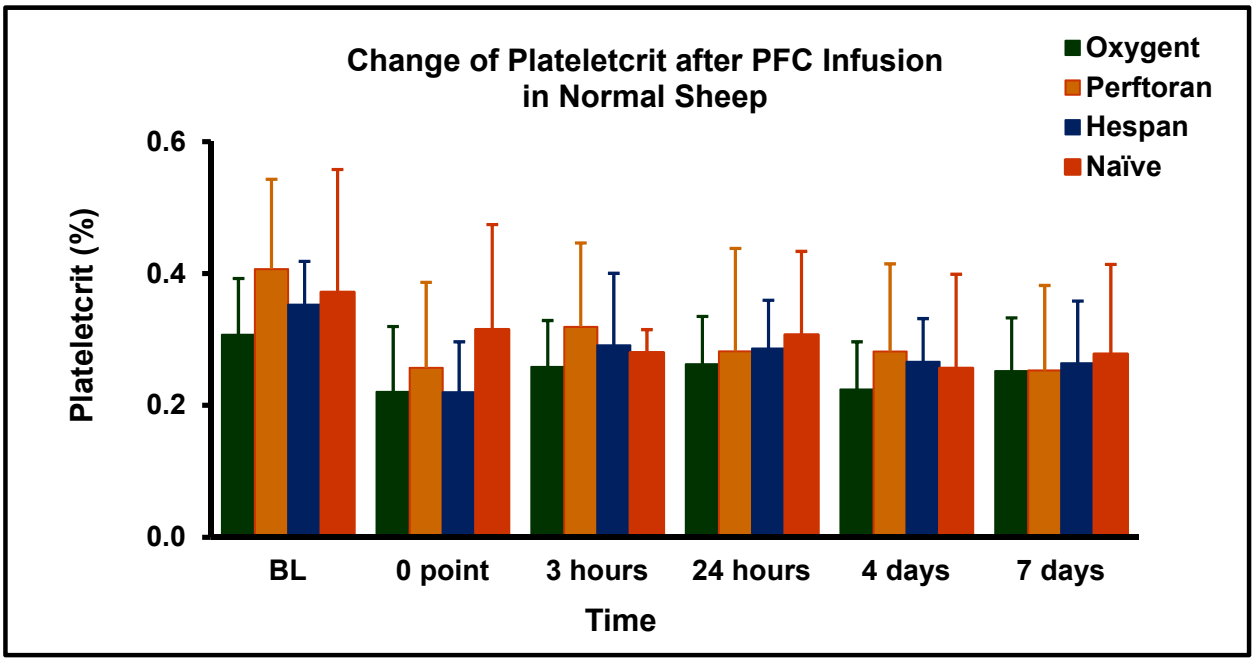


Figure 4 showed the changes of plateletcrit after PFC intravenous infusion compared with hespan infusion and naïve controls. Plateletcrit level in PFC groups did not show a significant difference compared with hespan group and naïve group. All groups showed a reduction (20%) as compared with their baseline right after infusion.

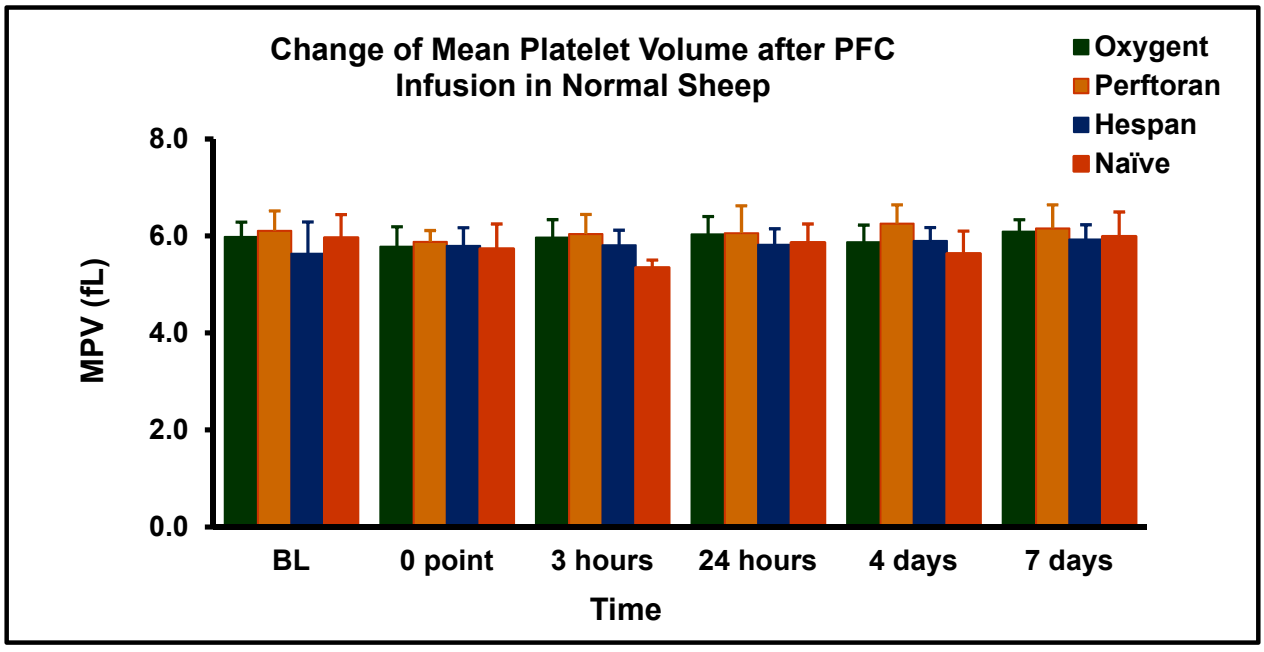


Figure 5 showed the changes of mean platelet volume (MPV) after PFC intravenous infusion compared with hespan infusion and naïve controls. MPV in PFC groups did not show a significant difference compared with hespan group and naïve group as well as compared with their baseline.

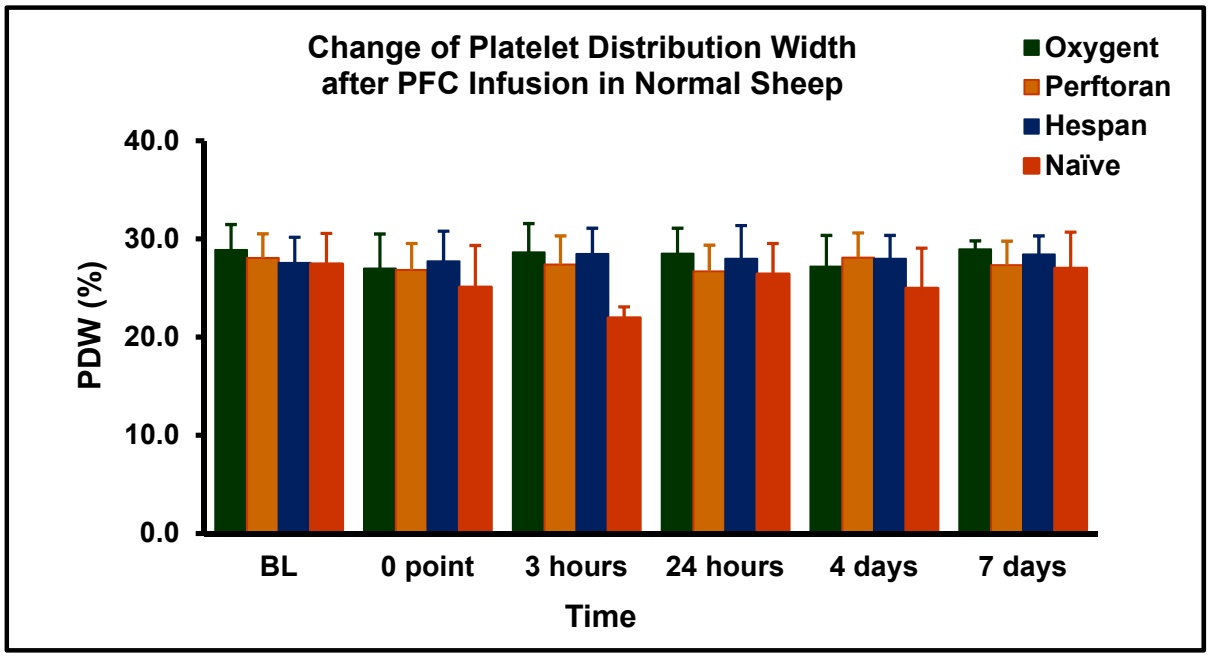


Figure 6 showed the changes of platelet distribution width (PDW) after PFC intravenous infusion compared with hespan infusion and naïve controls. PDW in PFC groups did not show a significant difference compared with hespan group and naïve group as well as compared with their baseline.

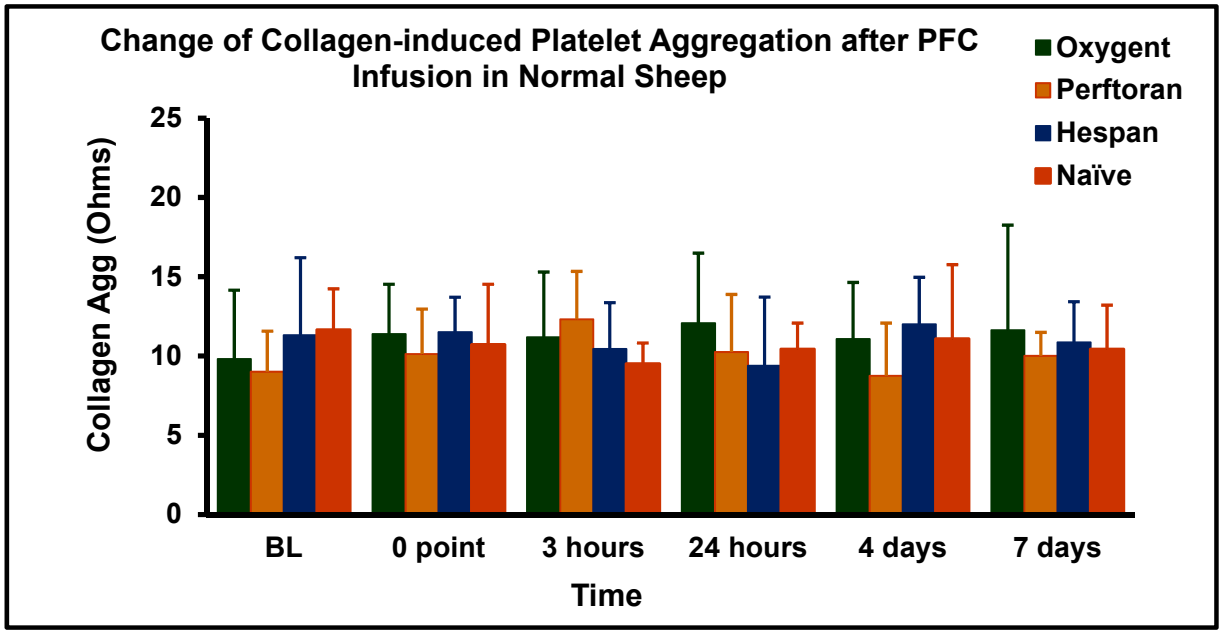


Figure 7 showed the effect of PFC infusion on collagen induced platelet aggregation. There were no significantly change in collagen induced platelet aggregation after PFC infusion compared with hespan infusion and naïve controls as well as as compared with their baseline.

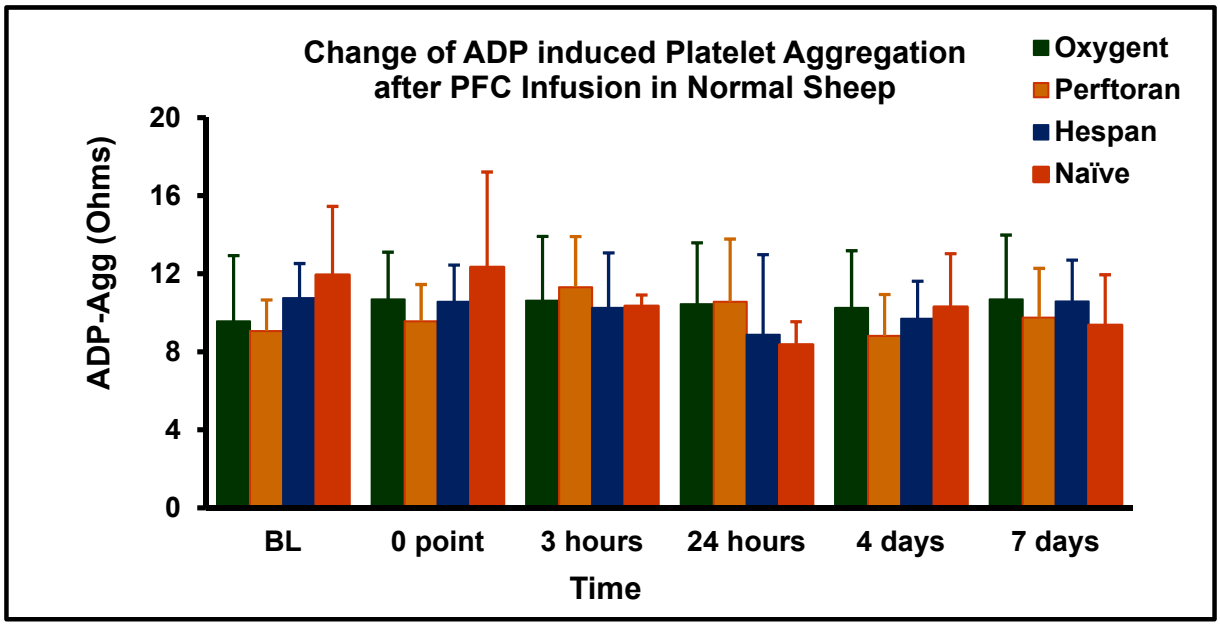


Figure 8 showed the effect of PFC infusion on ADP induced platelet aggregation. There were no significantly change in ADP induced platelet aggregation after PFC infusion compared with hespan infusion and naïve controls as well as as compared with their baseline.

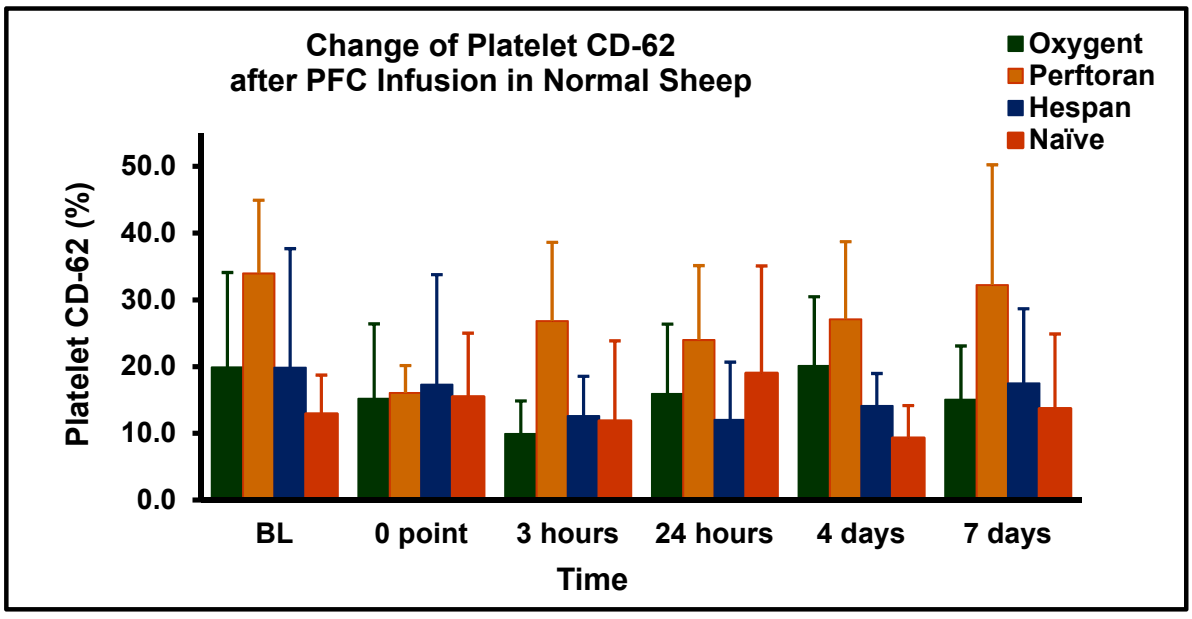


Figure 9 showed the effect of PFC infusion on platelet adhesion molecule CD62, which attached on the surface of activated platelets. PFC infusion did not increase CD62, which indicated that there was no increase platelet activation after PFC infusion.

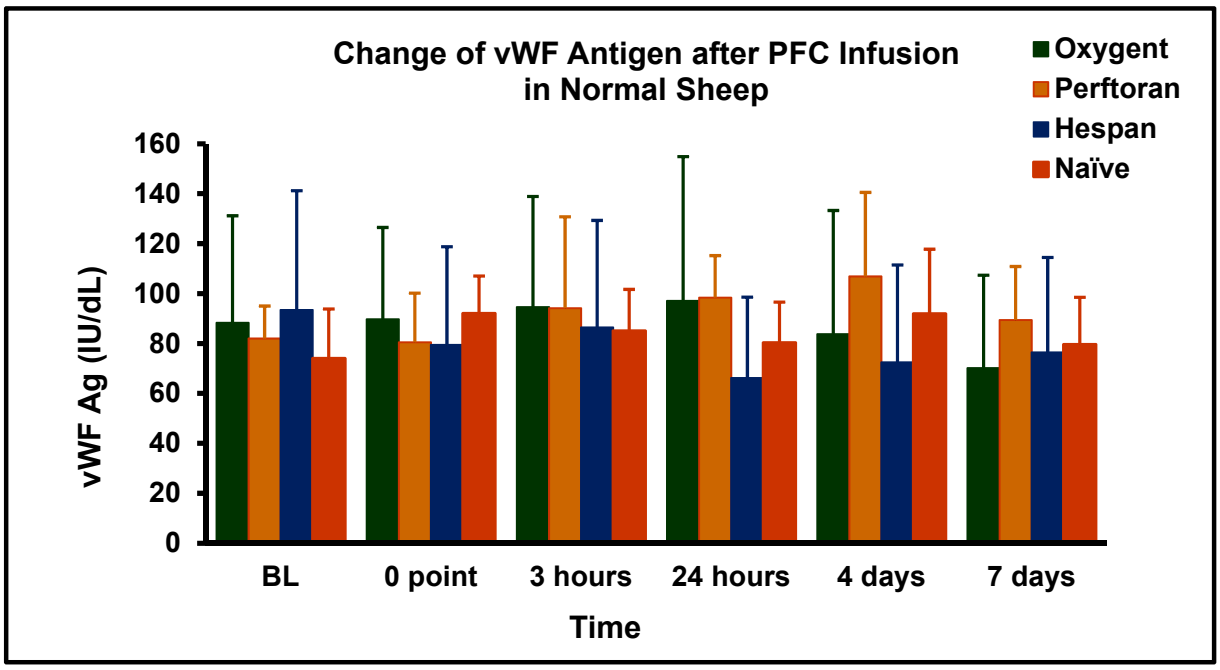


Figure 10 showed the effect of PFC infusion on vWF Ag (von Willebrand Factor Antigen) level. There were no significantly change in vWF Ag level (variation <15%) after PFC infusion compared with their baseline. There is no significant difference as compared with control groups at each survival time points.

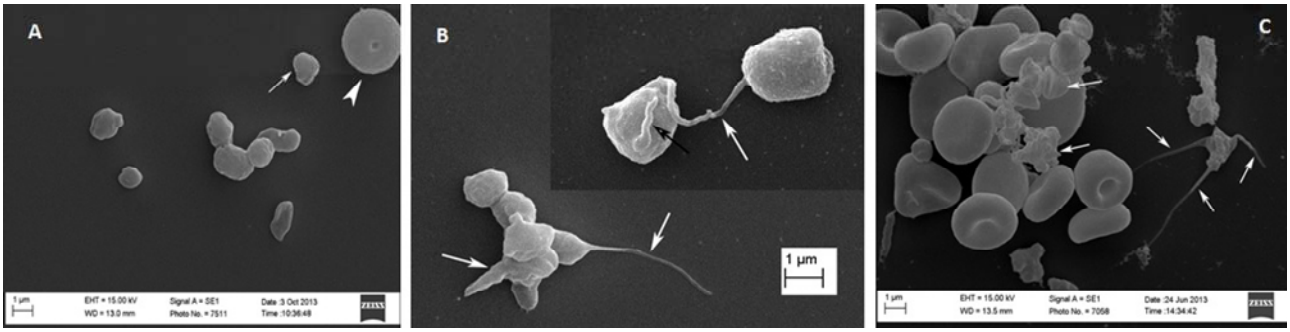


Figure 11. Platelet images of Scanning electron microscope. **A.** (on left) Non-active platelets (white arrow) and red blood cell (arrow head). Non-active platelets are small size with smooth surface. **B.** (middle) Semi-active platelets are with one or two processes (white or black arrows) and increase their size. **C** (on right) Active platelets are with 3 or more processes on surface (white or black arrows) and their surface becomes irregular or granular or conjugated together.

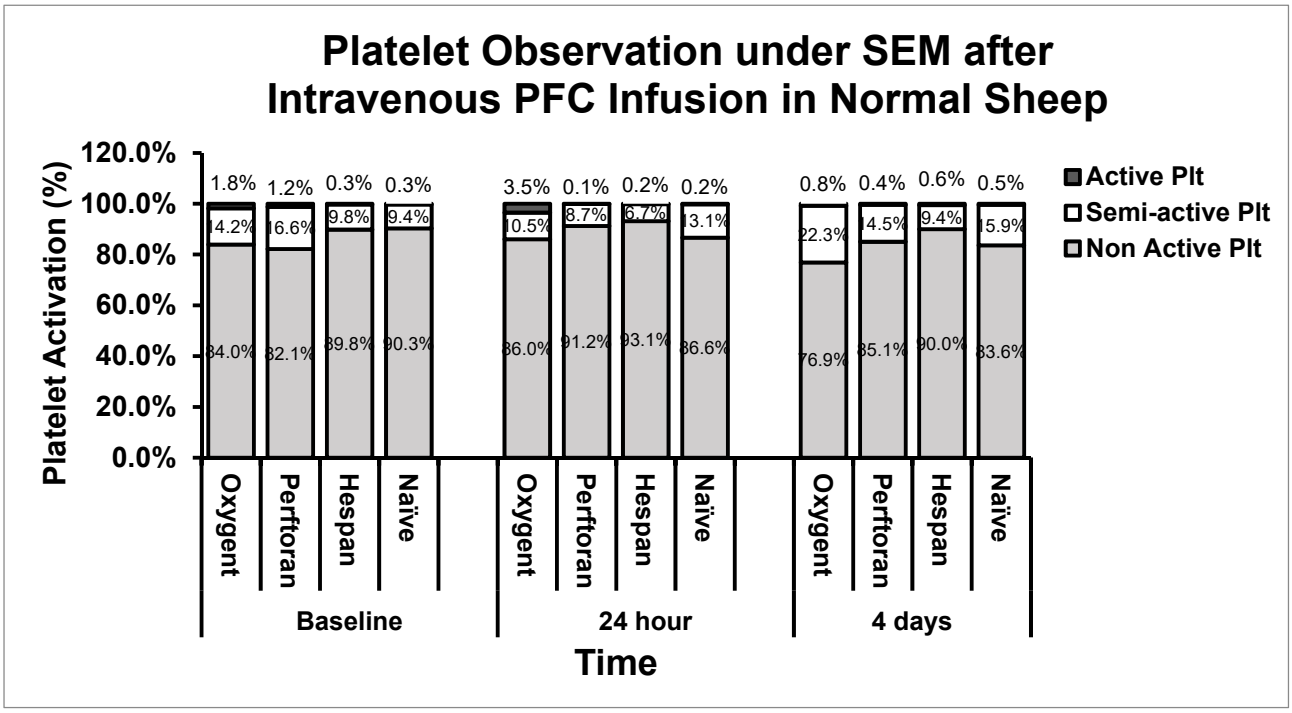


Figure 12. Based on the morphological characteristics, quantitative analysis results showed that PFC infusion did not significantly change the platelet activation as compared with their baseline or compared with control groups.

Task 1 Summary:

1. Platelet number is reduced transiently at first 24 hour following intravenous infusion of PFC (Oxygent and Perftoran) and hespan in health sheep.
2. PFC infusion did not change platelet aggregation nor change fibrinogen and vWF Ag levels as compared with hespan controls.
3. Quantitative Platelet morphological activation (observation with scanning electron microscopy) was correspond with the results of platelet functional assays, which indicates PFC infusion would not significantly activate or inhibit platelet function.

Task2: Using sheep (ovine, 20-30 kg) hemorrhagic shock model to test the effect of PFC intravenous infusion as an adjuvant resuscitation fluid on platelet number and activation. Animals were anesthetized, instrumented and had bleeding 35~50% of total blood volume and maintain mean arterial pressure at 30 mmHg (±3 mmHg) for 60 minutes then resuscitated with hetastarch plus PFC (Oxygent, Perftoran), hetastarch plus saline, and surgical control group, n=10/group. Venous blood samples were collected at baseline, 1 hour, 24, 96 and 168 hours (7 days) post PFC infusion for Plt/white cell activation as described in task1. Specific objectives included: **a)** development of a survival hemorrhagic shock model in sheep; **b)** measurement of platelet number and activation in hemorrhagic shock; **c)** status of coagulation factors in hemorrhagic shock (see appendix table1: list of measured parameters and table 3: summary results); **d)** exam the status of platelet activation with scanning electron microscope after PFC infusion comparing with saline infusion or sham control sheep, n= 10/group.

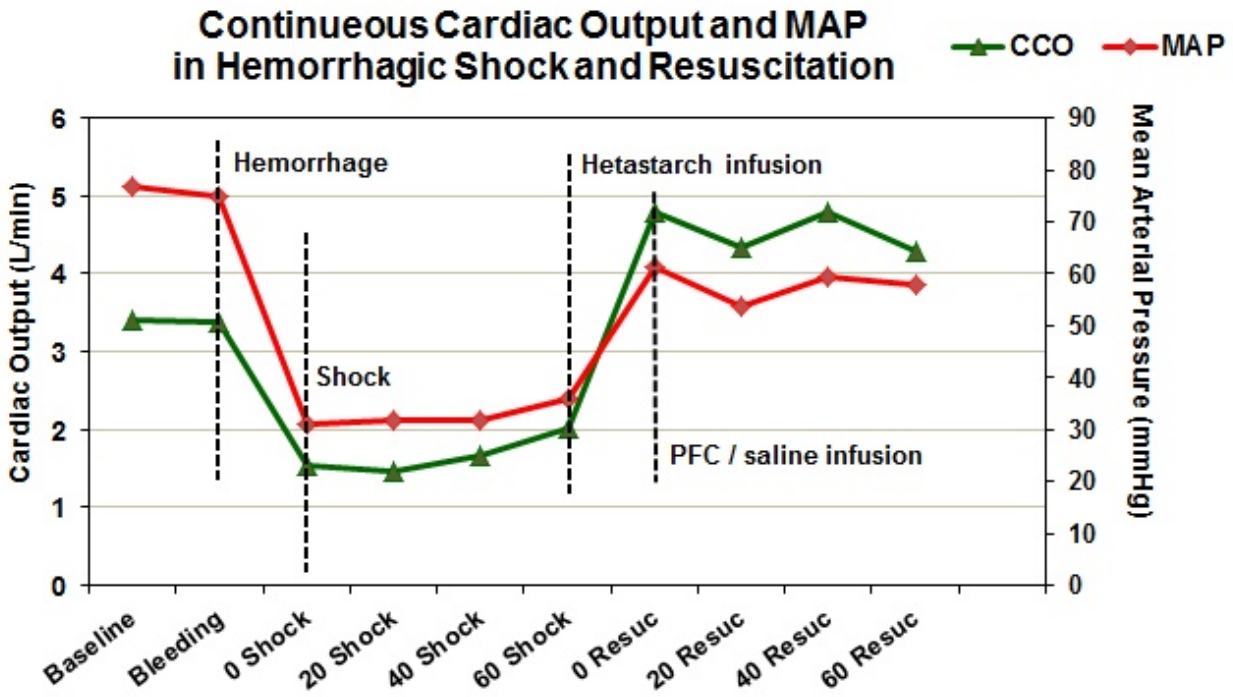


Figure 13. Sheep hemorrhagic shock model. Animals were anesthetized with isoflurane, instrumented and had bleeding 35~50% of total blood volume (62% body weight) and maintaining mean arterial pressure (MAP) at 30 mmHg (±3 mmHg) for 60 minutes then resuscitated with hetastarch first until MAP reach to 55 mmHg, then infusion PFC (Oxygent or Perftoran), or saline as controls. n=10/group.

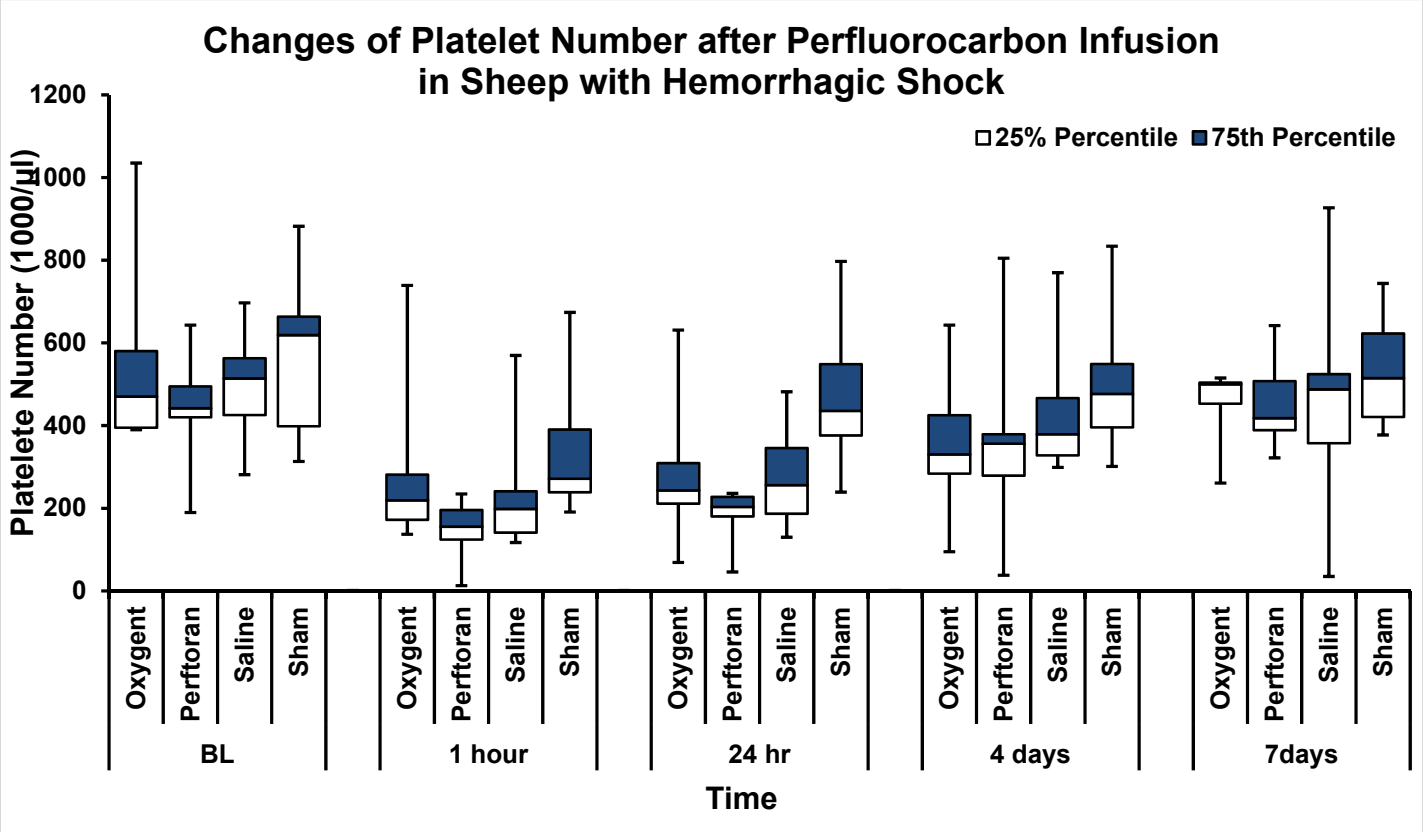


Figure 14 showed the changes of platelet numbers after PFC intravenous infusion as an adjuvant resuscitation fluid following the first resuscitation with hespan infusion. Platelet number was reduced in all hemorrhagic shock animals and sham animals at 1 hour compared with their baseline. Platelet number was reduced at 24 hours in all hemorragic shock animals as compared with their baseline and sham controls. PFC infusion did not show a significant difference compared with saline infusion animals. At 7 days, Platelet number did not show a significant difference among goups. The normal sheep platelet number is from 100,000 ~ 800,000 / microliter (mcL).

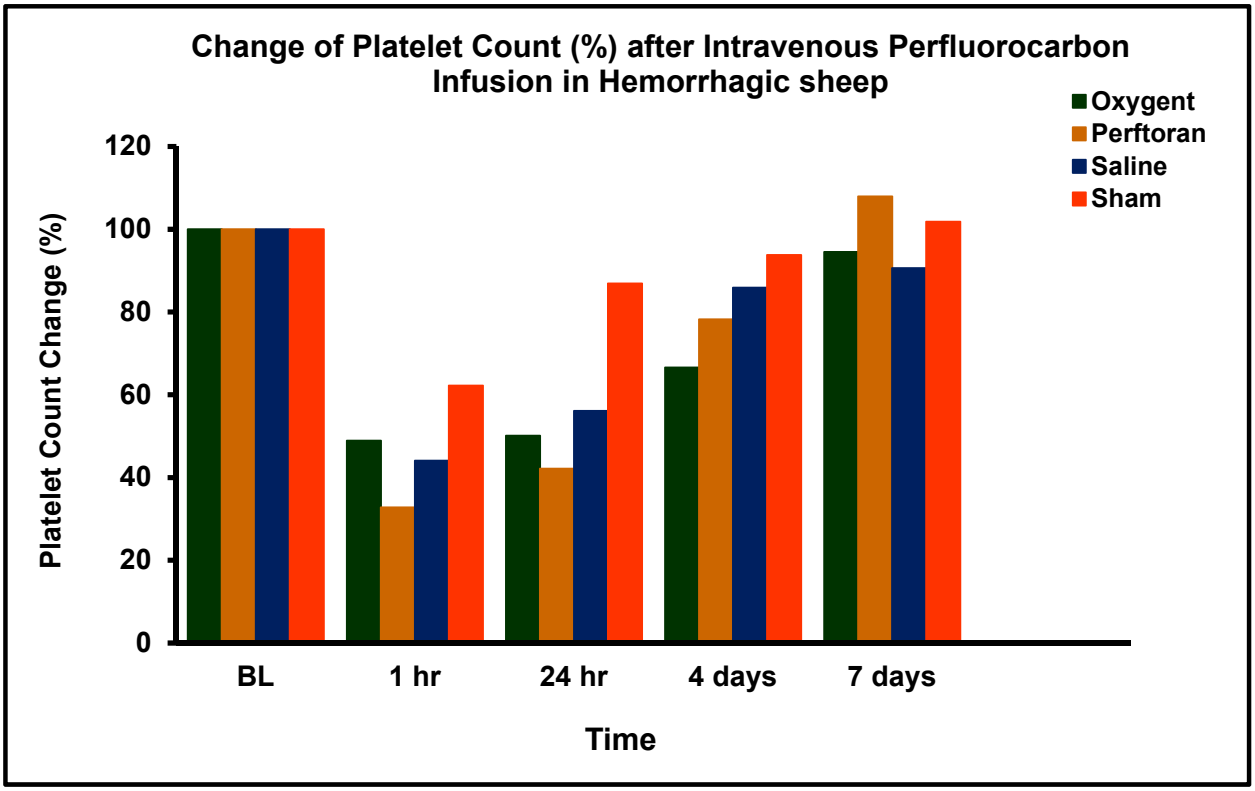


Figure 15 showed the percentage changes of platelet numbers after homorrhagic shock and non-blood fluid resuscitation. Platelet number was reduced at 1 hour after the resuscitation (40~60% reduction) in all group animals. Platelet number returned to near normal level at 24 hours in sham animals and at 7 days, there is no significant difference in platelet number among groups.

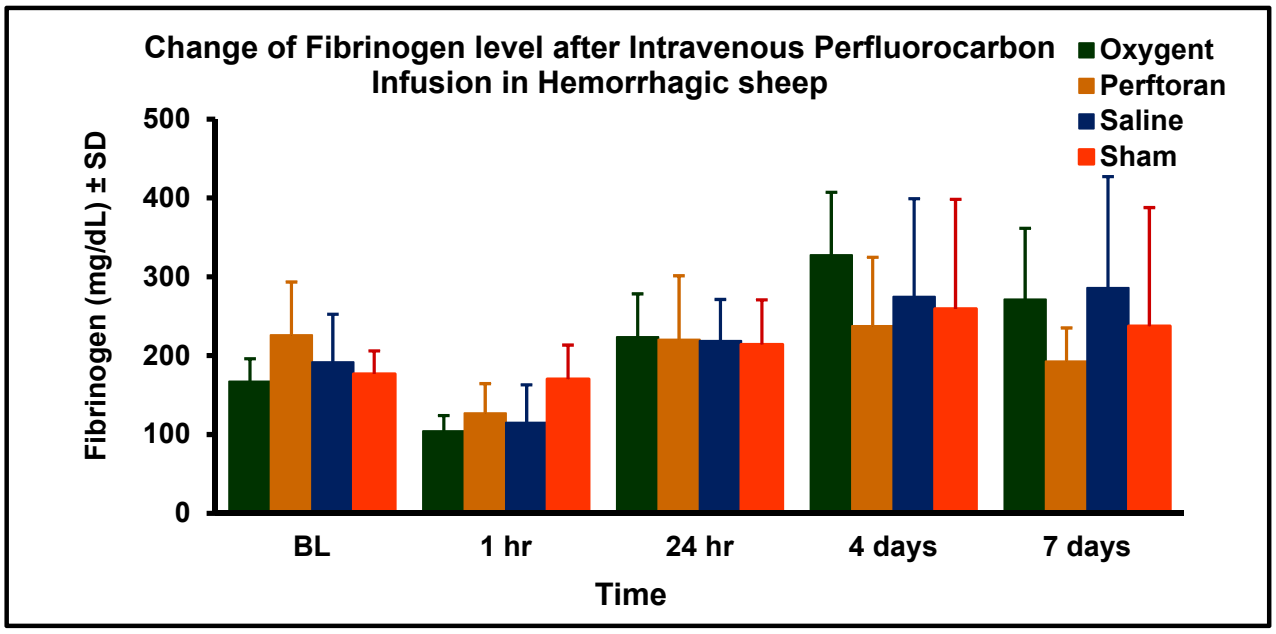


Figure 16 showed the changes of fibrinogen level after homorrhagic shock and non-blood fluid resuscitation. Fibrinogen level was reduced at 1 hour after the non-blood fluid resuscitation (40% reduction) in all hemorrhagic group animals compared with sham animals and returned to normal level at 24 hours as compared with their baseline as well as sham controls.

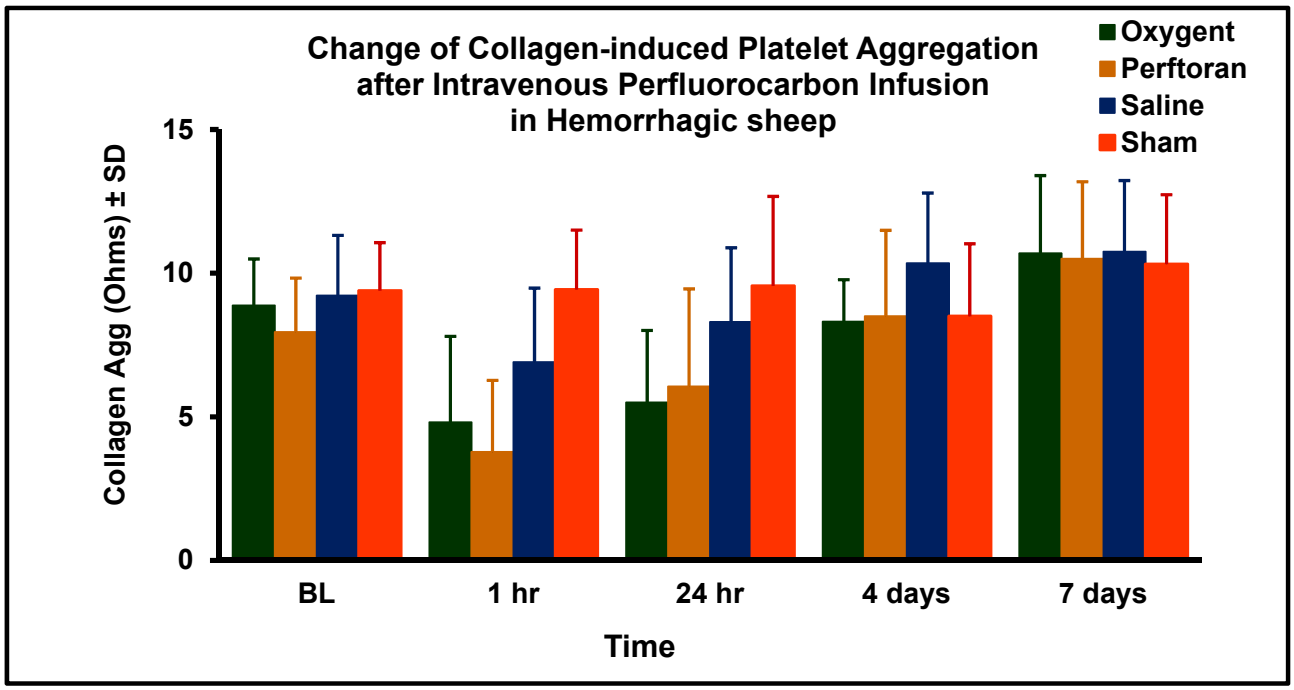


Figure 17 showed the effect of PFC infusion on collagen induced platelet aggregation. There was a significantly change in collagen induced platelet aggregation in PFC infusion groups at 1 hour and 24 hours post hemorrhagic shock compared with their baseline and sham controls. Collagen induced platelet aggregation of PFC groups returned to their baseline at 4 days post hemorrhagic shock.

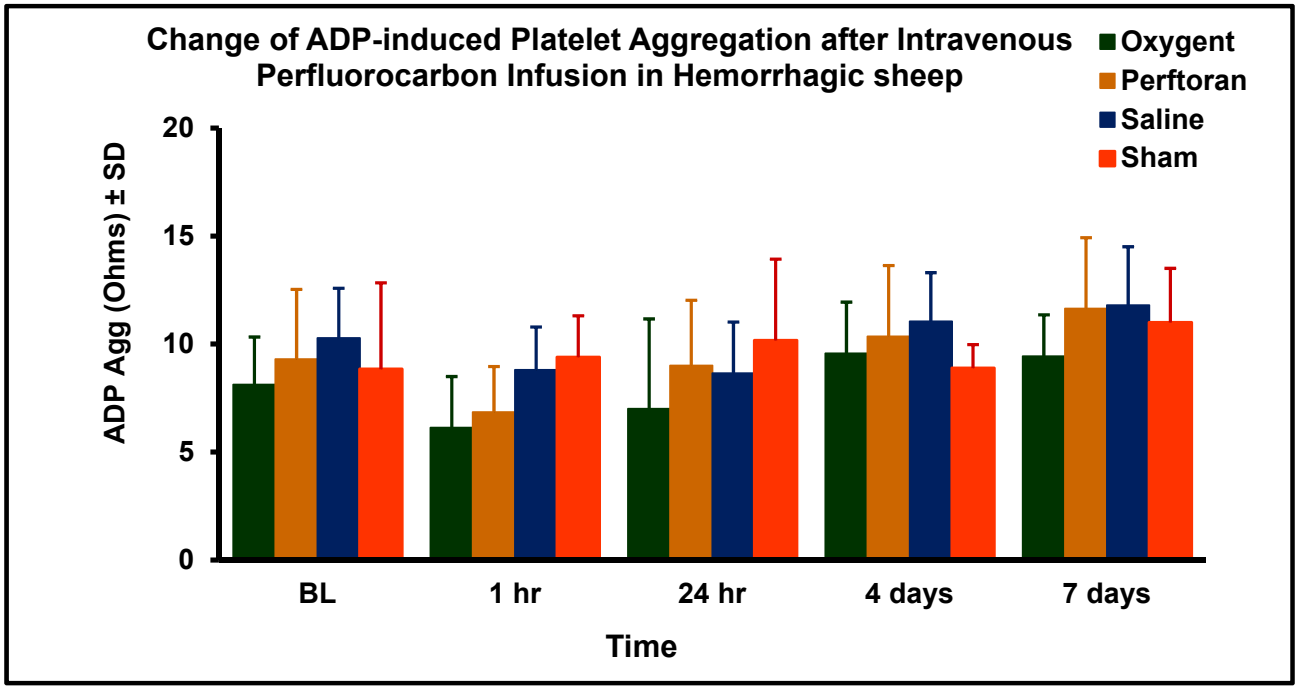


Figure 18 showed the effect of PFC infusion on ADP-induced platelet aggregation. There was a significantly change in collagen induced platelet aggregation in PFC infusion groups at 1 hour post hemorrhagic shock

compared with their baseline and the controls. ADP-induced platelet aggregation of PFC groups returned to their baseline at 4 days post hemorrhagic shock.

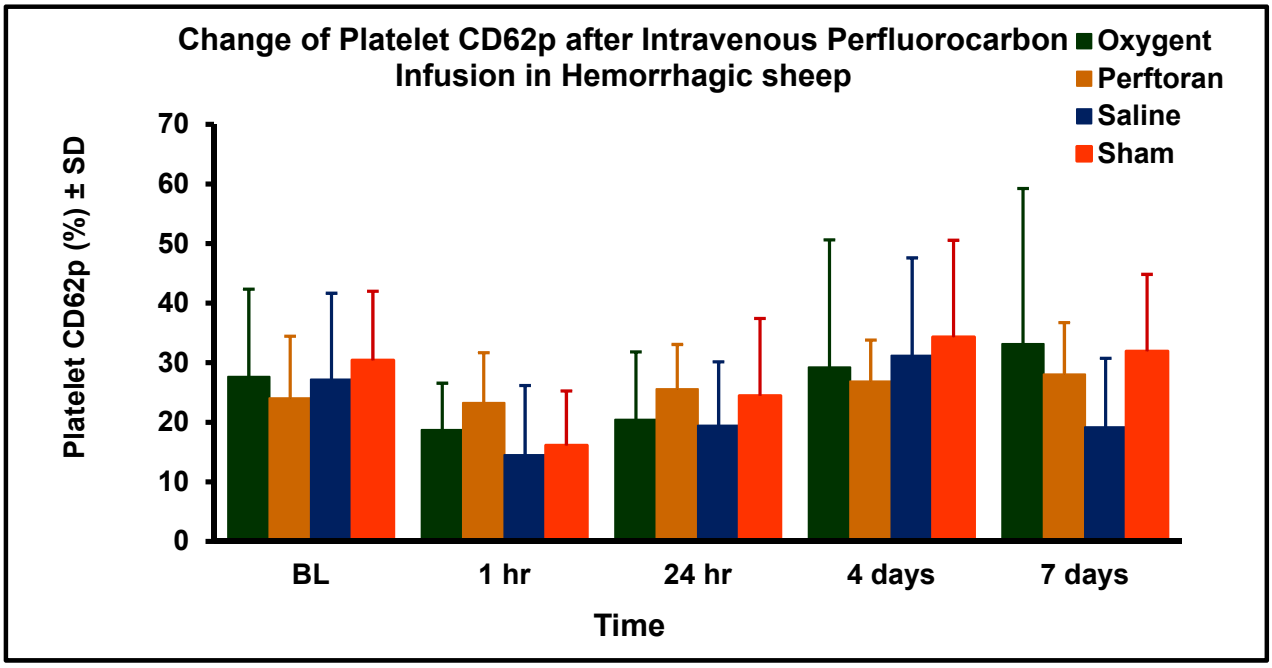


Figure 19 showed the effect of PFC infusion on platelet adhesion molecule CD62, which attached on the surface of activated platelets. Platelet CD62p showed a decrease at 1 hour after hemorrhagic shock with non-blood fluid resuscitation except perftoran group. Platelet CD62p returned to the baseline level at 24 hours (sham group) and at 4 days (all groups).

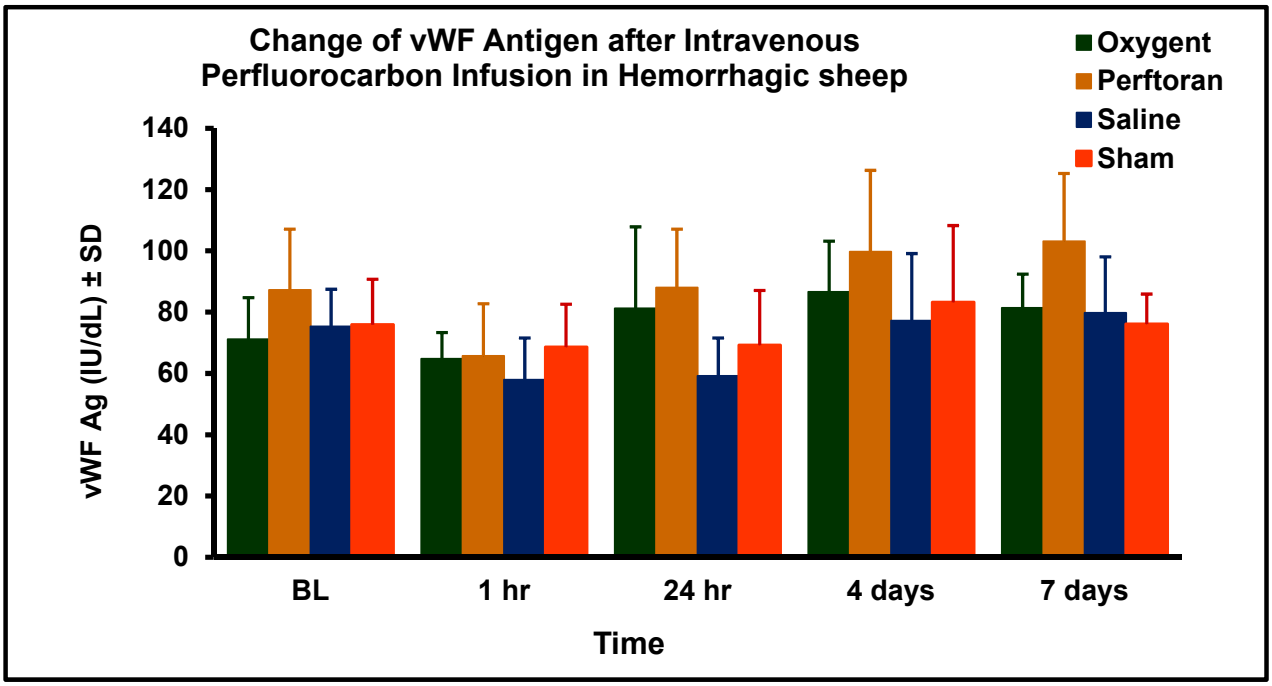


Figure 20 showed the effect of PFC infusion on vWF Ag (von Willebrand Factor Antigen) level in hemorrhagic shock sheep. There were no significantly change in vWF Ag level (variation <15%) after PFC infusion compared with their baseline. There is no significant difference as compared with sham groups at each survival time points.

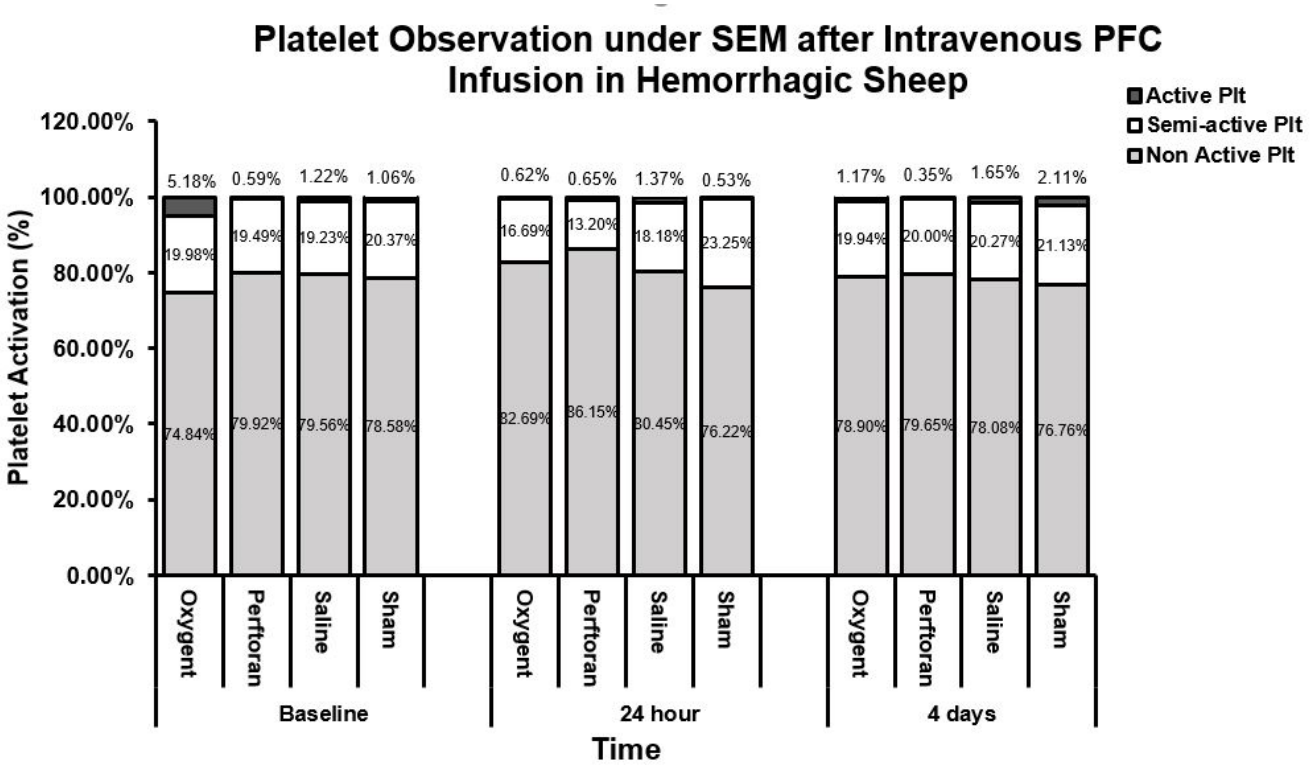


Figure 21. Based on the morphological characteristics as described in task1 (Figure 11), quantitative analysis results showed that PFC infusion did not significantly change the platelet activation as compared with their baseline or compared with control groups in hemorrhagic shock sheep.

Task 2 Summary:

1. A survival hemorrhagic shock sheep model (stepwise stage arterial bleeding total 35~50% blood volume, 62% of body weight) was successfully developed.
2. In hemorrhagic shock sheep, platelet number is significantly reduced at 1, 24 hours (50% decrease) and 4 days (20-30%) in PFC treated animals. Platelet number returns to the baseline at 7 day post hemorrhagic shock and resuscitation.
3. In hemorrhagic shock sheep, collagen or ADP induced platelet aggregations and platelet CD62p were not significantly difference among groups. PFC infusion did not change platelet function compared with controls.
4. Quantitative Platelet morphological activation (observation with scanning electron microscopy) was correspond with the results of platelet functional assays, which indicates that PFC intravenous infusion would not exaggerate coagulopathy in hemorrhagic shock animals.

Task3: Using the sheep polytrauma model of the blast traumatic injury (over pressure trauma) following hemorrhagic shock to test the effect of PFC (Perftoran) intravenous infusion on platelet number and activation. Volume resuscitation will occur with either hetastarch with saline or hetastarch with PFC. Study endpoints were carried out as described in Task1 and Task2. Specific objectives included: a) measurement of platelet number and activation in sheep polytrauma model; b) status of coagulation factors in sheep polytrauma model (see appendix

table1: list of measured parameters and table 4: summary results); c) exam the status of platelet activation with scanning electron microscope after PFC infusion comparing with saline infusion or sham control sheep, n= 10/group.

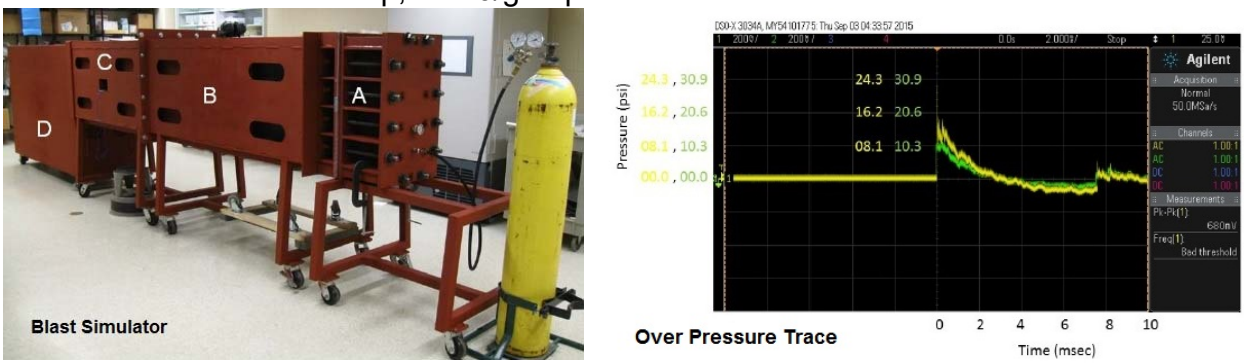


Figure 22 showed a blast simulator (left side) and over pressure trace (right side). Sheep polytrauma model was subjected to blast trauma first following hemorrhagic shock.

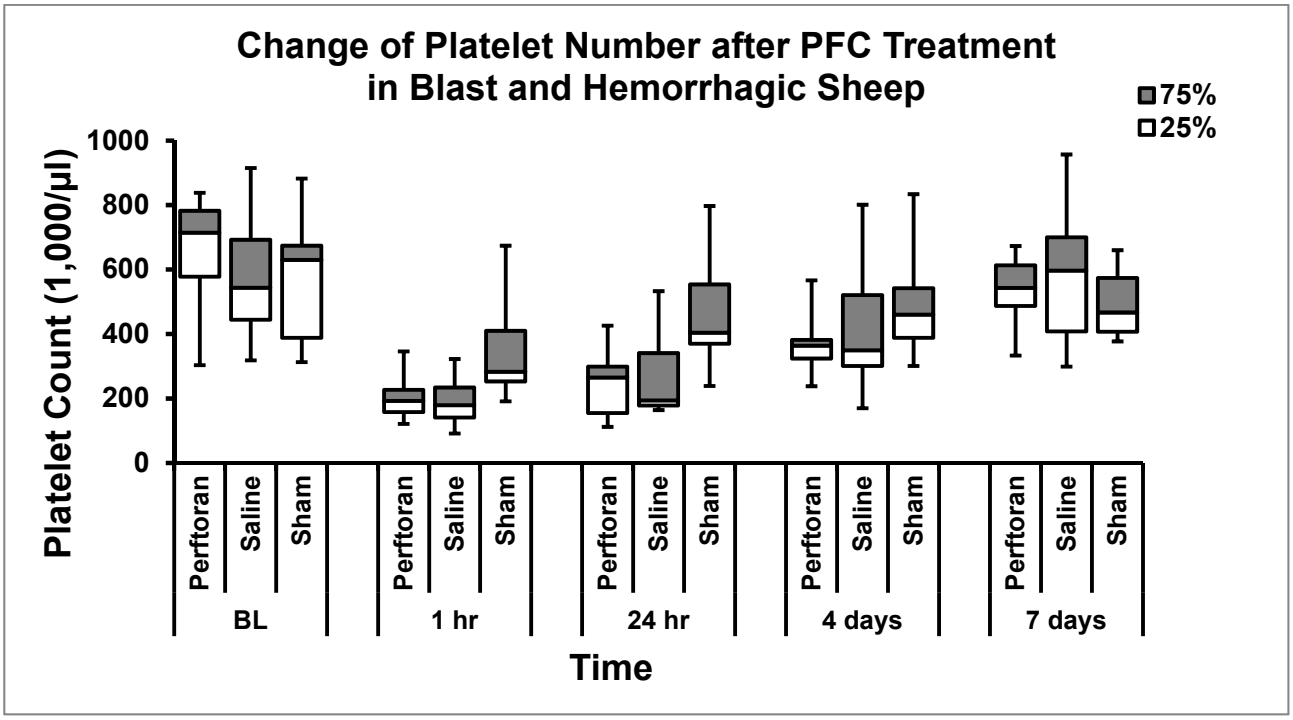


Figure 23. In normal sheep, platelet mean value is about 400,000/µl (range from 100,000 to 800,000). The results showed that platelet counts were significantly decreased at 24 hour after PFC or saline resuscitation compared with the sham group or compared with their own baselines. However, there were no significant changes between PFC and saline groups at any time points before or post resuscitation. Platelet number returned back to baseline level at 7 days post-injury. Platelet count and mean distribution with standard error. Box plot shows platelet count distribution with minimum value, maximum value, 25%, median, and 75% values.

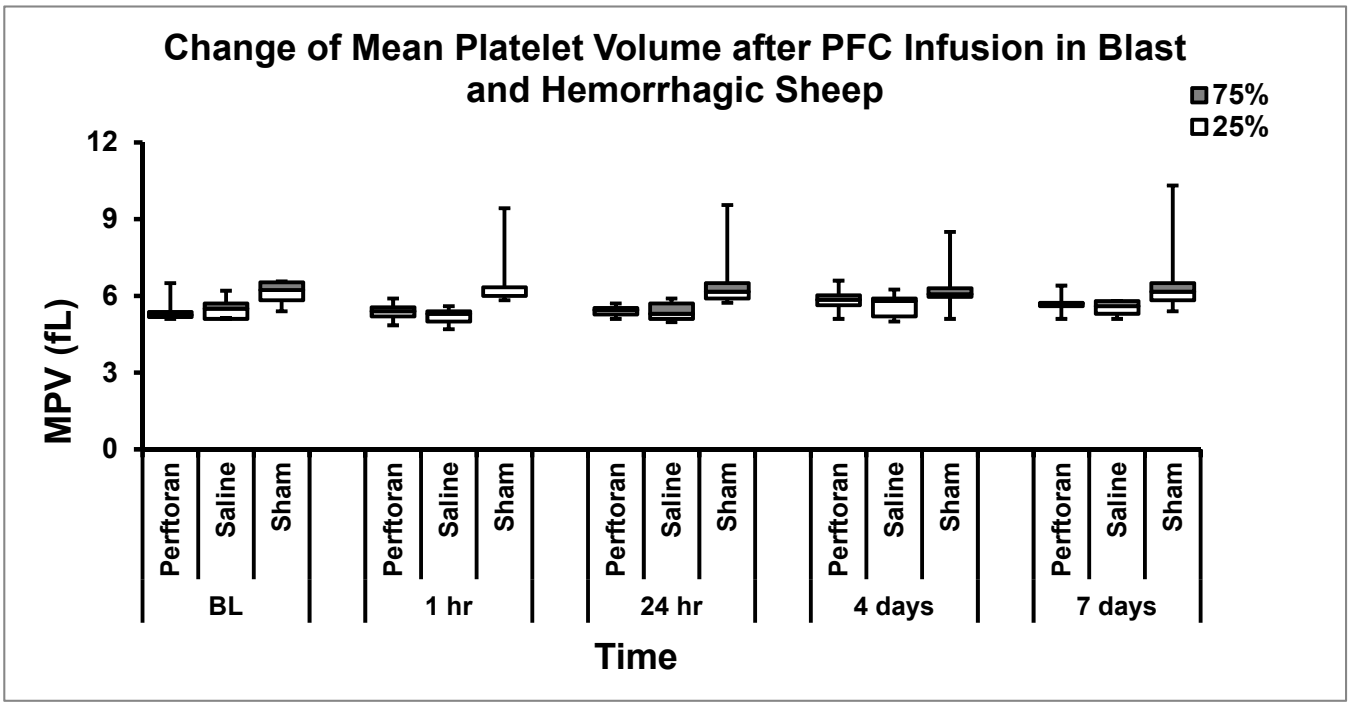


Figure 23. **Mean platelet volume (MPV)** did not show significant changes among groups, which indicated that the decrease platelet number might be the result of non-blood fluid resuscitation (hemodilution).

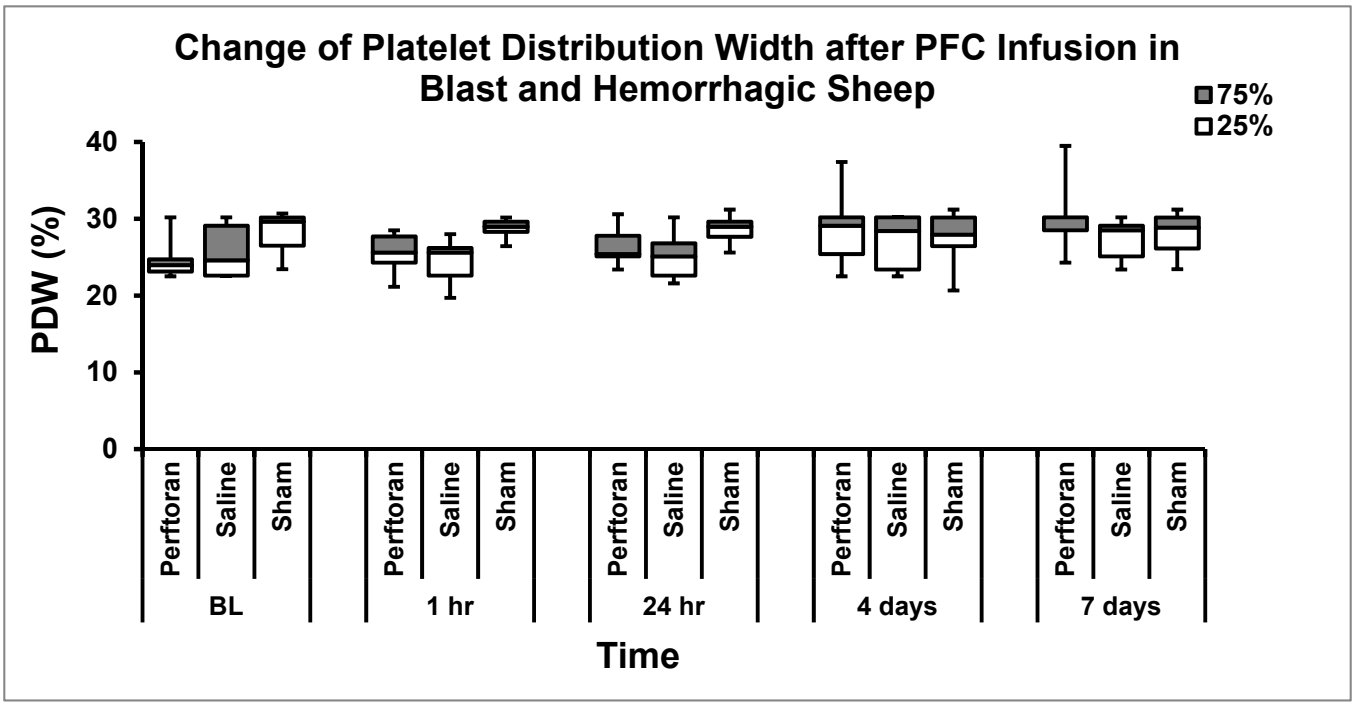


Figure 24. **Platelet distribution width (PDW)** did not show significant changes among groups, which indicated that the decrease platelet number might be the result of non-blood fluid resuscitation (hemodilution).

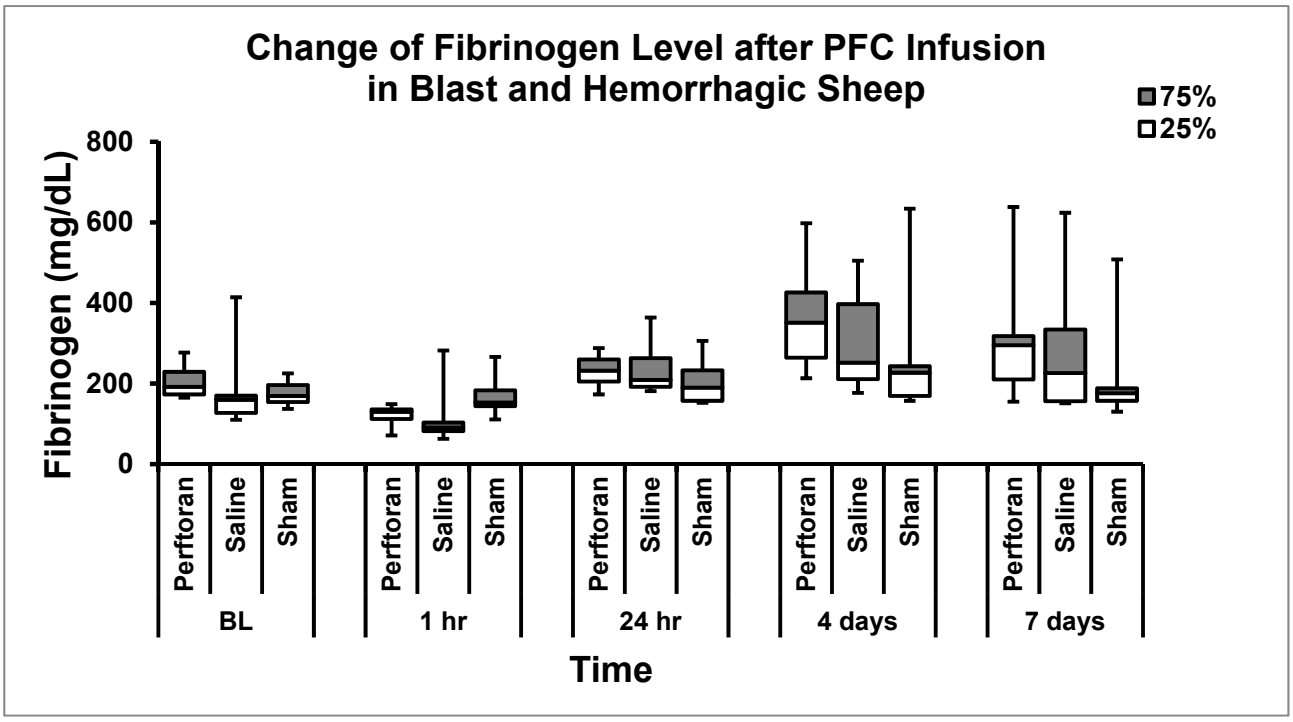


Figure 25. **Fibrinogen** level showed a decrease at one hour in injured groups (PFC and Saline groups and returned to baseline level at 24 hours. There is no significant difference when the groups are compared each other at 24 hours and 4 & 7 days indicating no massive clot formation after injury and treated with PFC.

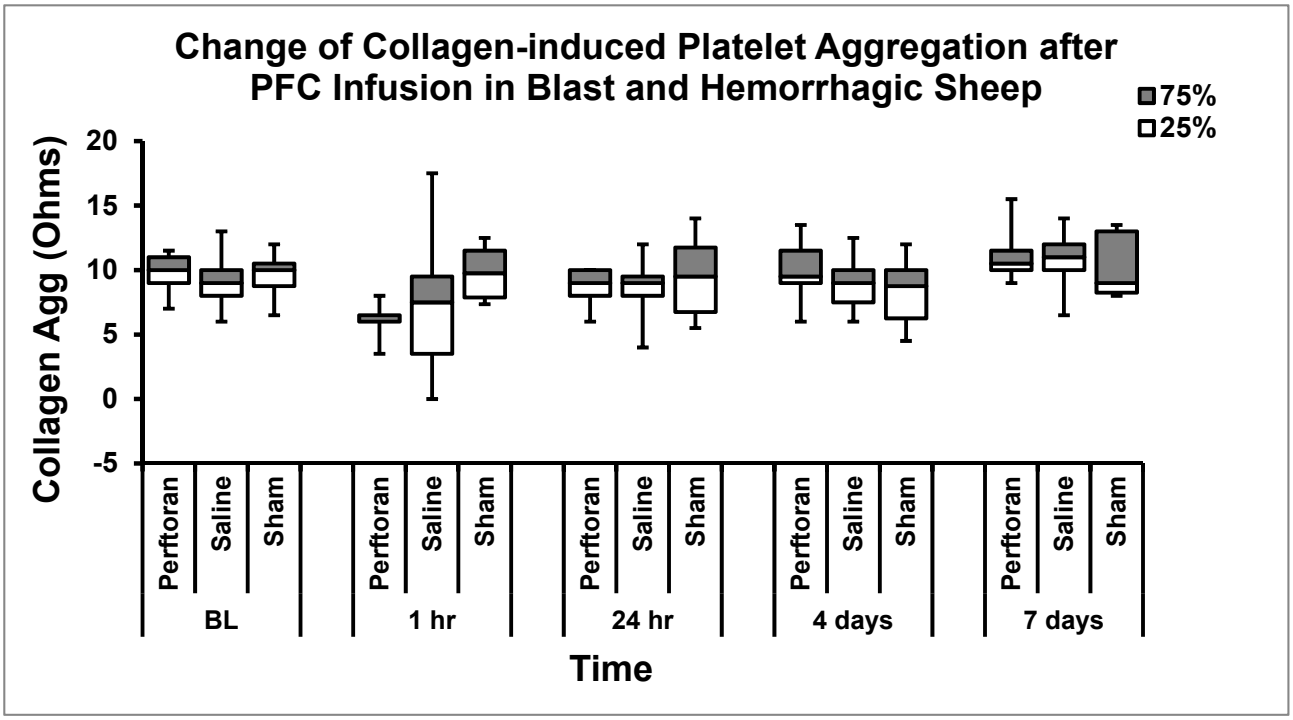


Figure 26. Platelet aggregation testing measures the ability of various agonists to platelets to induce in vitro activation and platelet-to-platelet activation. Collagen induced platelet aggregation (strong agonist, Collagen-Agg) Collagen-Agg was significantly decreased at 1 hour after resuscitation and returned back to baseline level at 24 hours post-resuscitation. There were no significant changes between PFC and saline groups at any time points before or post resuscitation indicating that PFC treatment after trauma/hemorrhage did not make platelet function worse in the current injury model.

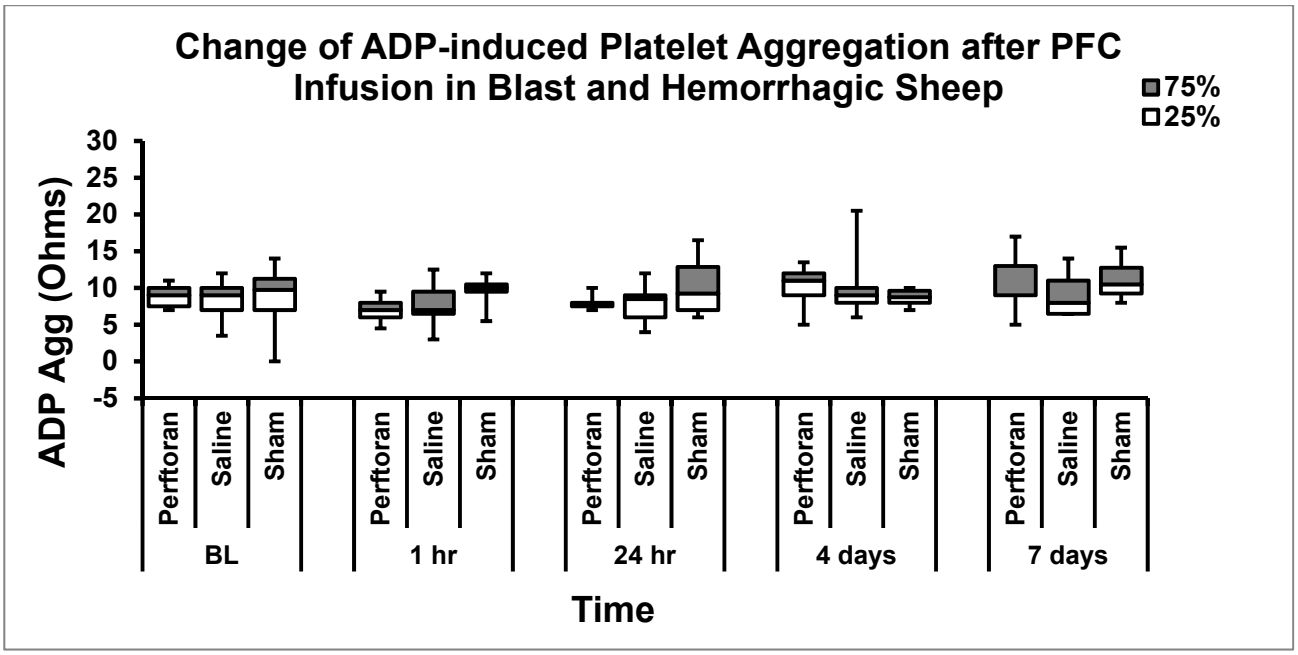


Figure 27. Platelet aggregation testing measures the ability of various agonists to platelets to induce in vitro activation and platelet-to-platelet activation. ADP induced platelet aggregation (weak agonist, ADP-Agg). The data showed that ADP-Agg was significantly decreased at 1 hour after resuscitation and returned back to baseline level at 24 hours post-resuscitation. There were no significant changes between PFC and saline groups at any time points before or post resuscitation indicating that PFC treatment after trauma/hemorrhage did not make platelet function worse in the current injury model.

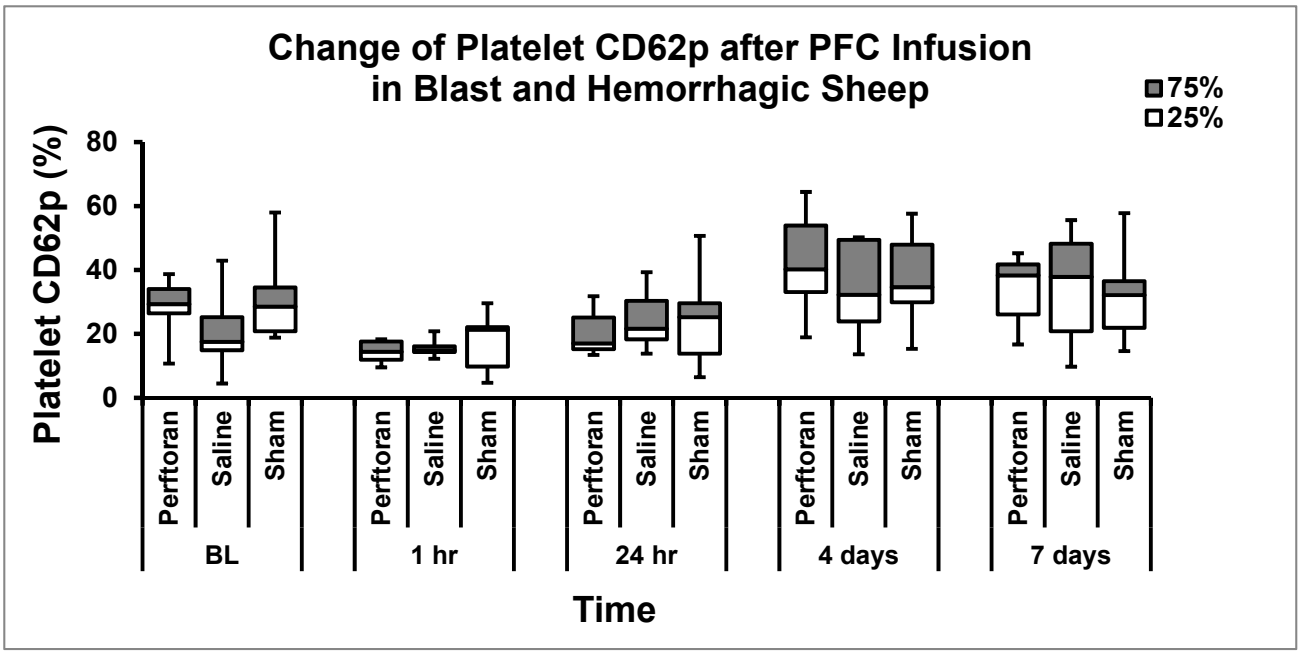


Figure 28. Platelet CD62p (platelet surface expression of CD62P (P-selectin), Fig 12) was significantly decreased at 1 hour and did not show significant changes between PFC and saline groups. Platelet CD62p expression returned to baseline and increased expression at 4 days and 7 days at post-resuscitation compared with baseline.

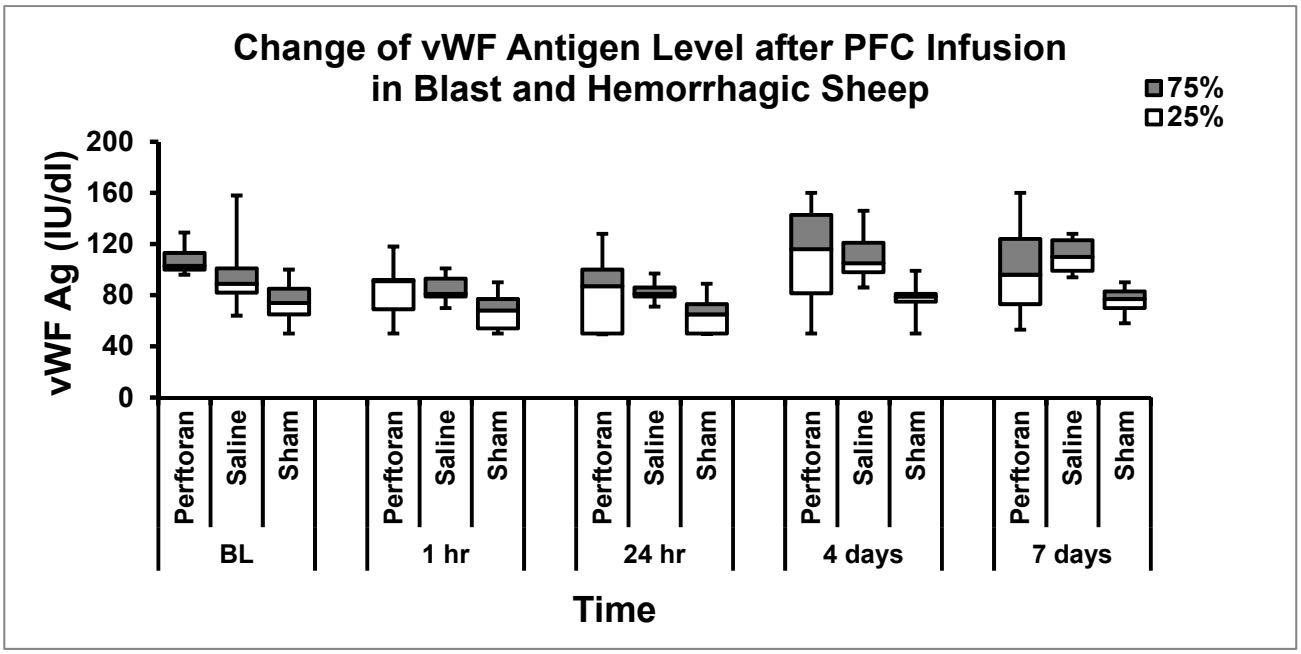


Figure 29. von Willebrand Factor Antigen (Factor VIII:R Antigen) is important for platelet-platelet and platelet-vessel hemostatic interactions. The data showed that vWF Ag decreased at one hour in PFC and Saline groups and returned to the baseline level at 24 hours. There is no significant difference when the groups were compared each other at 24 hours and 4 & 7 days. Platelet function assessment results indicate that intravenous PFC treatment did not significantly change platelet function using combined blast and hemorrhagic shock sheep model.

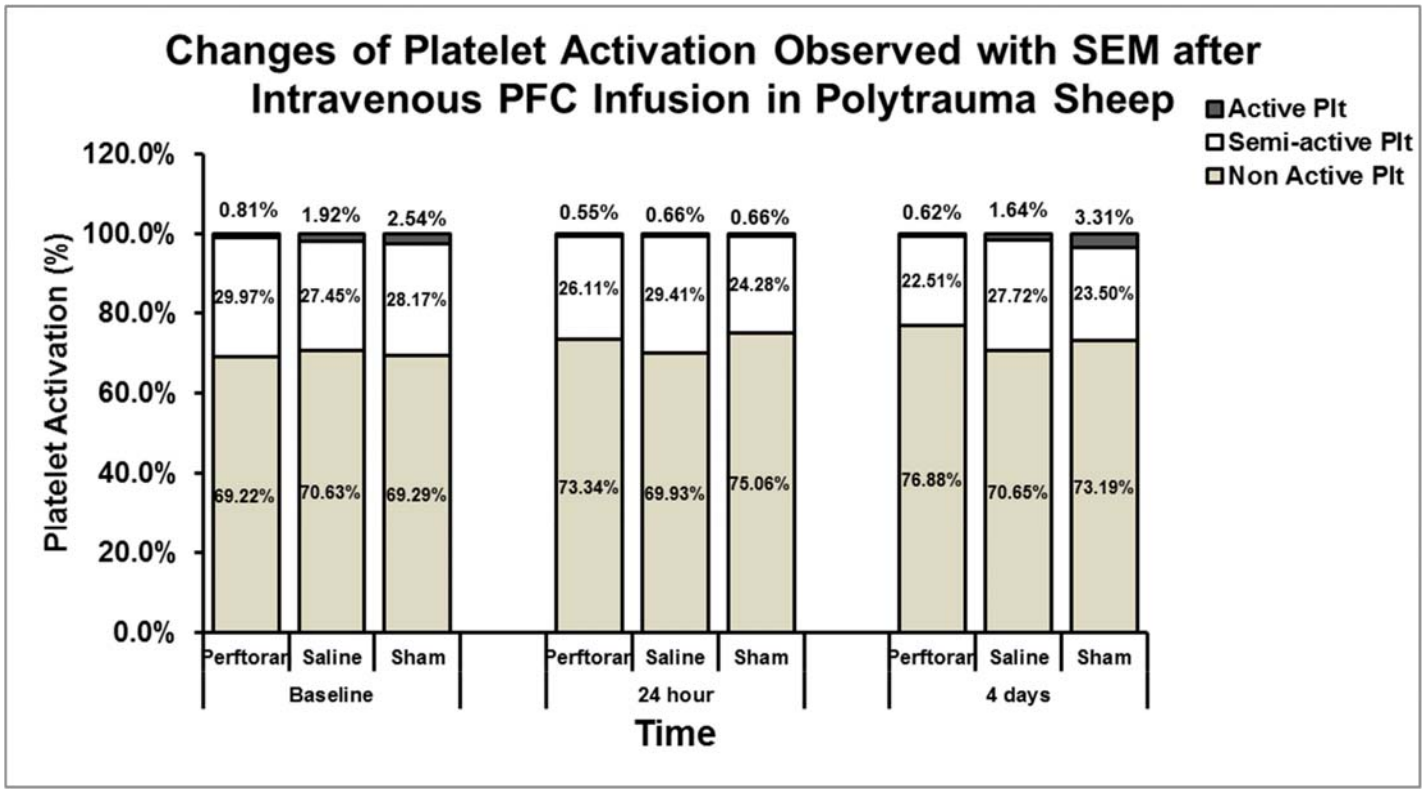


Figure 30. Based on the morphological characteristics as described in task1 (Figure 11), quantitative analysis results showed that PFC infusion did not significantly change the platelet activation as compared with its baseline or compared with control groups (saline and sham) in the blast trauma and hemorrhagic shock sheep.

Task 3 Summary:

1. A survival polytrauma model was successfully development, which combined blast trauma with hemorrhagic shock in sheep.
2. In combined blast trauma with hemorrhagic shock sheep, platelet number is significantly reduced at 1, 24 hours and 4 days in PFC and saline treated animals. Platelet number returns to the baseline at 7 day post injury and resuscitation. There is no significant difference in platelet number between PFC and saline (positive control) animals. The results suggested that the reduction of platelet number may be more related with the injury and the non-blood fluid resuscitation instead of PFC infusion.
3. In polytrauma sheep, platelet function assay showed that collagen or ADP induced platelet aggregations and platelet CD62p were not significantly difference among groups. PFC infusion did not change platelet function compared with controls.
4. Quantitative Platelet morphological activation (observation with scanning electron microscopy) was correspond with the results of platelet functional assays, which indicates that PFC intravenous infusion would not exaggerate coagulopathy in the polytrauma animals.

Opportunities for training and professional development has the project provided:

Students and fellows were trained in this project (2013-2016)

Name	Status	Time	Project
J. Mark Hylton	Medical Student	June-July 2013; March 2016	Image analysis of Scanning electron microscope
Kyle Flores	Medical Student	June-July 2013	Coagulation factors
Matt Hallman	Medical Student	June-July 2013	Biomarkers of blast trauma
Michael Waters	MD/PhD student	Mar 2013-June 2014	Brain bleeding analysis of deep brain stimulation model, using lab facility and guide animal handling
Evan Kraus	Medical Student	June-July, 2014	Image and data analysis of Scanning electron microscope
Viktoras Palys	MD, Resident	2014-2015	Brain bleeding analysis of deep brain stimulation model, using lab facility and guide animal handling
Paul Middleton	MD, resident research	July 2014-2016	Non-invasive cardiac output measurement using Cheetah device
Brandon Janssen	Medical Student	June-July, 2015	Hemodynamics of hemorrhagic shock
Suneel Thummala	MD, resident research	March 2015-2016	Blood and CSF sample biomarkers analysis of hemorrhagic shock and polytrauma

How were the results disseminated to communities of interest?

Some of the results have been presented on Military Health System Research Symposium (MHSRS) conferences (2014-2016). The data will be published on several scientific journals and will submitted to FDA as support evidence for the further PFC research and clinical trial.

4. IMPACT:

A sheep hemorrhagic shock survival model was successfully established, which can be used for many other studies such as testing long term outcome of resuscitation (blood substitute infusion) in hemorrhagic shock and enroute critical care.

Current results indicate that the change of platelet number is subject to several factors including resuscitation fluid infusion such as hespan (hetastarch) and perfluorocarbon emulsions. Platelet number is subject to hemorrhagic shock injury.

Current project indicate that PFC infusion would not exaggerate or lead platelet malfunction in the normal sheep and in the hemorrhagic shock sheep. These data are encouraging for FDA approval of further clinical trial study of PFC in the United States.

Technology for PFC intravenous infusion,

1) **Slow infusion:** intravenous infusion perfluorocarbon emulsions must be given in a slow speed to allow PFC to be dissolved in blood (Oxygent with 60% PFC, 3 gram/kg or 5 ml/kg, infusion over 15 minutes). Fast infusion of PFC may cause pulmonary congestion because undissolved PFC could block the pulmonary microcirculation. Therefore, PFC infusion should be carried out under a good systemic circulatory condition.

2) **PFC Shelf-life:** current product's shelf life is about 2 years indicated by manufacture vendor. However, during pre-clinical animal studies, repetitive open / close and re-refrigeration of PFC package could cause an acceleration of phospholipid emulsion breakdown, which would exaggerate the side effects of PFC. For future studies, GMP (Good Manufacturing Practice) product (PFC) should be required.

3) **Reduce isoflurane dosage during PFC infusion:** PFC increases gas solubility including inhale anesthetics (30%) (Cuignet et al., 2002; Mecozzi et al., 2008). Therefore, isoflurane dosage should be re-adjusted to avoid overdose when PFC infusion is ongoing.

5. CHANGES/PROBLEMS:

Two major problems we had met during this project.

First, the laboratories used for the project were experienced flood on 24 November 2013 and many experimental devices were damaged. Relocation laboratories twice (moved out after flood and moved back after renovation) and replacement of damaged equipment were taken for several months, which delayed the progress of the project.

Second problem was delayed manufacture of perfluorocarbon by vendor. We had used Oxygent for the project and tried to order more PFC at the second year of the study but

we received the product in March 2016, the date closed to the end of the project. Based on the original design, we should test another PFC, PHER-O2 (88% w/v PFC, produced by Sanquine Corporation Inc.), but we could not order the product and have to switch to test Perftoran (Russian PFC formula). Because of delayed manufacture of Oxygent, we could not complete to exam the effect of Oxygent on platelet number and function in sheep polytrauma model.

6. PRODUCT

Scientific Conference Presentation List:
Oral presentation:

2013,Jun: *Studies of the Effects of Perfluorocarbon Emulsions on Platelet Number and Function in Models of Critical Battlefield Injury*. U.S. Army Hemorrhage and Resuscitation Research and Development Program, Metabolic and Tissue Stabilization Research In-Progress Review, Fort Dietrich, MD

Conference / Meeting presentation

1. 2016 International Anesthesia Research Society Annual meeting (May 19-24, 2016): Paul A. Middleton, MD, Penny S. Reynolds, PhD, Jiepei Zhu, MD PhD, Bruce D. Spiess, MD. Evaluation of Noninvasive Cardiac Output Monitoring in Sheep with Hemodynamic Instability

2. 2016,Aug, Military Health System Research Symposium: **Parsons, J.T.**, Thummala, S.K., McCarter, J.R., Sweeney, C.R., Middleton, P.A., Zhy, J., Spiess, B.D. Temporal Analysis Of Biomarkers Of Brain Damage In Ovine Survival Models Of Hemorrhage And Blast / Hemorrhage Polytrauma With Perfluorocarbon Treatment. MHSRS-16-1514.

3. 2016,Aug, Military Health System Research Symposium: Zhu, J., **Parsons, J.T.**, Martin, E.K., McCarter, J.R., Sweeney, C.R., Middleton, P.A., Thummala, S.K., Berger, B.B., Mohammed, B.M., Brophy, D., Spiess, B.D. The Effect of Adjunctive Perfluorocarbon Infusion on Platelet Number and Function in a Blast / Hemorrhage Polytrauma Sheep Model. MHSRS-16-1009.

4. 2015,Sept, Congress of Neurological Surgeons: Palys, V., Lotz, D.T., Waters, M., Zhu, J., Holloway, K.L., **Parsons, J.T.** Intracerebral hematoma incidence in a coagulopathic sheep model of deep brain stimulation (DBS) surgery: microelectrode versus DBS electrode penetration.

5. 2015,Aug, Military Health System Research Symposium: Zhu, J., **Parsons, J.T.**, Martin, E.K., McCarter, J.R., Sweeney, C.R., Middleton, P.A., Berger, B.B., Kraus, E.J., Brophy, D., Spiess, B.D. Effect of Intravenous Perfluorocarbon on Platelet Number and Function in Hemorrhagic Sheep. #2022

6. 2015,Aug, Military Health System Research Symposium: Hallman, M., Flores, K., McCarter, J., Sweeney, C., Morris, A., Zhu, J., Spiess, B.D., **Parsons, J.T.** The Effect of Perfluorocarbon Oxygen Therapeutics in a Sheep Survival Model of Severe Hemorrhagic Shock. #2041

7. 2015,Jun, VCUHS Resident Research Day: Palys, V., Lotz, D.T., Waters, M., Zhu, J.,

Holloway, K.L., **Parsons, J.T.** Brain parenchyma penetration using either blunt-tipped or sharp-tipped hardware

a. seems to cause the similar rate of hemorrhage in sheep model. #49

8. 2015 May, Virginia Commonwealth University, Student Research Honor Day: Evan Kraus*, Jiepei Zhu, J. Travis. Parsons, Erika J. Martin, Jacquelyn McCarter, Christopher Sweeney, Paul Middleton, Donald Brophy, Bruce D. Spiess, Effect of Intravenous Perfluorocarbon on Platelet Number and Function in Hemorrhagic Sheep. (* First year medical student, Dean's research fellowship winner)

9. 2014,Aug, Military Health System Research Symposium: Zhu, J., **Parsons, J.T.**, McCarter, J.R., Sweeney, C.R., Hylton, Jr., J.M., Martin, E.K., Brophy, D., Spiess, B.D. Effect of Perfluorocarbon on Platelet Number and Function after Intravenous Infusion in Sheep

10. 2014,May VCU SOM Medical Student Honors Day: Flores, K., Hallman, M., Leonard, K., Sweeney, C., Zhu, J., McCarter, J., Spiess, B.D., **Parsons, J.T.** Effects of Perfluorocarbon Therapy on Cerebellar Alpha-2 Spectrin Breakdown Products in Repetitive Blast Traumatic Brain Injury

11. 2014,May VCU SOM Medical Student Honors Day: Hylton, Jr., J.M., **Parsons, J.T.**, McCarter, J., Sweeney, C. Spiess, B.D., Zhu, J. Morphological Characteristics of Platelets Post Perfluorocarbon Emulsion Infusion

Scientific Publication Preparation List:

1. Effect of Perfluorocarbon Infusion on Platelet Number in Normal Male Juvenile Sheep
2. Ultrastructure observation of Platelet Activation in Normal and Hemorrhagic Shock Sheep after Perfluorocarbon Infusion: Scanning and Transmission Electronic Microscopic Analysis
3. Effect of Perfluorocarbon Infusion on Platelet Activation in Normal Male Juvenile Sheep: ROTEM Assay Analysis
4. Effect of Perfluorocarbon Infusion as A Part of Resuscitation Fluid on Platelet Number and Activation in Hemorrhagic Sheep
5. Evaluation of Noninvasive Cardiac Output Monitoring in Sheep with Hemodynamic Instability

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Personnel	Role	Percent Effort
Bruce D. Spiess, MD	PI (Dec. 2012-April 2016)	20%
Jiepei Zhu, PhD	Co-investigator, animal experiment PI (April 2016-June 2016)	75%
J. Travis Parsons, PhD	Co-investigator, blast injury, biomarker analysis	80%
Donald Brophy, Pharm.D	Co-Investigator, Coagulation lab	5% (subaccount)
Brian Berger, BA	Lab manager	35%
Jacquelyn McCarter	Technician, animal experiment	100%
Christopher Sweeney, BA	Technician, animal experiment	100%
Paul A. Middleton, MD	Fellow, animal experiment, data analysis	hourly
Suneel Thummala, MD	Master student, Biochemistry sample analysis	hourly
Penny S. Reynolds, PhD	Data analysis (2015-2016)	10%
Andrew Morris, BA	Animal experiment (2013-2014)	50%
Erika J. Martin, MT	Director of Coagulation laboratory	subaccount

The project was approval for non-cost extension from 14 December, 2015 to 14 June, 2016.

Principal investigator Dr. Bruce Spiess started his new position at the department of anesthesiology, university of Florida on 1 May 2016 and Dr. Jiepei Zhu was approved for the principal investigator until the end of the project.

8. SPECIAL REPORTING REQUIREMENTS:

Nothing to report

9. APPENDICES:

Table 1. List of Parameters

Table 2. Summary data sheet of task1

Table 3. Summary data sheet of task2

Table 4. Summary data sheet of task3

Biomarkers results

Sheep Hemorrhagic shock procedures

Abstracts of MHSRS and National Conference

Table 1. List of Parameters

Parameter	Baseline	0.0h	3h	24h	4d	7d
Natem CT (sec)	√	√	√	√	√	√
Natem CFT (sec)	√	√	√	√	√	√
Natem MCF (mm)	√	√	√	√	√	√
Intem CT (sec)	√	√	√	√	√	√
Intem CFT (sec)	√	√	√	√	√	√
Intem (MCF)	√	√	√	√	√	√
Extem CT (sec)	√	√	√	√	√	√
Extem CFT (sec)	√	√	√	√	√	√
Extem MCF (sec)	√	√	√	√	√	√
PCF (kdynes)	√	√	√	√	√	√
CEM (kdynes/cm^2)	√	√	√	√	√	√
FOT (min)	√	√	√	√	√	√
ETP (Nm• min)	√	√	√	√	√	√
Thrombin (nM)	√	√	√	√	√	√
Fibrinogen	√	√	√	√	√	√
Platelet Count	√	√	√	√	√	√
vWF:Ag	√	√	√	√	√	√
Collagen Agg (Ohms)	√	√	√	√	√	√
ADP Agg (Ohms)	√	√	√	√	√	√
Coll/Epi CT (sec)	√	√	√	√	√	√
Coll/ADP CT (sec)	√	√	√	√	√	√
CD41 (MFI)	√	√	√	√	√	√
CD62p (%)	√	√	√	√	√	√
PAC-1 (%)	√	√	√	√	√	√
CD45 (MFI)	√	√	√	√	√	√
CD11b (%)	√	√	√	√	√	√
CD62L (%)	√	√	√	√	√	√
CD61 (%)	√	√	√	√	√	√
PLT SEM	√			√	√	

Abbreviations: CT: clotting time; CFT: clot formation time; MCF: maximum clot firmness; PCF: platelet contractile force; CEM: clot elastic modulus; FOT: force onset time; ETP: endogenous thrombin potential; vWF:Ag: von Willebrand's factor antigen; Agg: platelet aggregations; Coll/Epi CT: Collagen/Epinephrine Closure Time; Coll/ADP CT: Collagen/ADP Closure Time;

Table 2: Summary data of Task1

0Parameter	Baseline		0 Hour		3 Hour		24 Hour		96 Hour		7 Days	
TOPLOAD	**Mean**	*SE*	**Mean**	*SE*	**Mean**	*SE*	**Mean**	*SE*	**Mean**	*SE*	**Mean**	*SE*
NATEM CT (sec)	**680**	*112.03*	**483**	*39.51*	**567**	*72.98*	**688**	*112.27*	**691**	*109.47*	**699**	*101.33*
	427	*65.42*	**603**	*44.55*	**439**	*71.94*	**504**	*71.30*	**590**	*75.69*	**482**	*58.28*
	587	*98.92*	**636**	*106.63*	**586**	*62.16*	**596**	*93.61*	**631**	*58.89*	**507**	*87.19*
	843	*124.17*	**752**	*73.86*	**715**	*140.32*	**836**	*126.79*	**914**	*157.52*	**844**	*166.34*
NATEM CFT (sec)	**195**	*32.07*	**121**	*18.01*	**139**	*13.34*	**161**	*20.01*	**194**	*45.96*	**216**	*43.18*
	122	*20.12*	**190**	*14.76*	**141**	*25.32*	**127**	*16.73*	**199**	*30.80*	**167**	*32.27*
	141	*23.77*	**220**	*40.44*	**153**	*21.12*	**153**	*23.32*	**176**	*16.87*	**152**	*39.28*
	232	*44.26*	**227**	*23.43*	**242**	*49.58*	**253**	*35.47*	**296**	*52.87*	**267**	*65.60*
NATEM MCF (mm)	**73.5**	*1.81*	**74.6**	*1.22*	**73.8**	*1.37*	**77.3**	*1.57*	**75.5**	*2.51*	**74.9**	*1.55*
	79.5	*1.32*	**72.4**	*1.36*	**76.0**	*1.65*	**76.5**	*0.93*	**73.0**	*1.44*	**73.1**	*2.70*
	77.9	*1.51*	**74.5**	*2.08*	**76.4**	*1.45*	**77.3**	*1.44*	**74.3**	*1.28*	**75.1**	*3.14*
	76.9	*2.35*	**75.3**	*1.91*	**73.0**	*3.29*	**75.4**	*2.03*	**70.5**	*2.76*	**73.5**	*2.79*
NATEM Angle (%)	**59**	*4.05*	**69**	*2.63*	**66**	*2.13*	**64**	*2.76*	**61**	*5.21*	**58**	*4.54*
	69	*3.26*	**57**	*1.88*	**66**	*3.71*	**67**	*2.57*	**58**	*3.41*	**62**	*4.69*
	67	*3.39*	**57**	*5.32*	**65**	*2.96*	**65**	*3.24*	**61**	*2.01*	**65**	*5.00*
	57	*5.45*	**56**	*2.84*	**53**	*6.12*	**55**	*2.81*	**49**	*5.21*	**54**	*6.27*
INTEM CT (sec)	**151**	*14.76*	**131**	*18.10*	**150**	*10.83*	**159**	*13.08*	**146**	*22.14*	**158**	*5.60*
	149	*13.09*	**208**	*7.88*	**157**	*15.60*	**155**	*14.93*	**164**	*11.89*	**159**	*9.86*
	156	*15.81*	**168**	*16.64*	**156**	*11.54*	**157**	*15.03*	**155**	*8.14*	**333**	*181.23*
	161	*18.22*	**168**	*11.52*	**167**	*14.64*	**184**	*11.58*	**177**	*12.73*	**166**	*10.28*
INTEM CFT (sec)	**56**	*3.74*	**69**	*5.12*	**61**	*4.36*	**57**	*3.34*	**52**	*3.94*	**50**	*5.45*
	48	*2.51*	**63**	*4.42*	**53**	*4.40*	**51**	*3.61*	**57**	*5.56*	**64**	*8.82*
	48	*2.70*	**65**	*6.21*	**61**	*8.22*	**59**	*3.40*	**66**	*4.39*	**62**	*4.55*
	60	*12.91*	**61**	*5.99*	**62**	*12.34*	**56**	*5.97*	**62**	*8.64*	**60**	*6.55*
INTEM MCF (mm)	**77**	*1.40*	**76**	*0.81*	**77**	*1.15*	**79**	*0.93*	**79**	*1.03*	**79**	*1.65*
	81	*0.84*	**78**	*0.96*	**80**	*1.57*	**80**	*1.03*	**79**	*1.28*	**76**	*1.74*
	80	*1.93*	**80**	*2.05*	**79**	*1.47*	**81**	*1.41*	**77**	*1.76*	**76**	*2.22*
	81	*1.56*	**80**	*1.97*	**78**	*2.75*	**80**	*1.88*	**78**	*2.18*	**79**	*1.84*
INTEM Angle (%)	**79.3**	*0.56*	**76.3**	*0.98*	**78.4**	*0.68*	**78.8**	*0.75*	**79.6**	*0.86*	**79.9**	*1.04*
	80.4	*0.56*	**77.0**	*0.87*	**79.3**	*0.94*	**79.5**	*0.73*	**79.3**	*1.26*	**77.5**	*1.51*
	76.6	*4.13*	**77.3**	*1.25*	**78.0**	*1.63*	**78.5**	*0.68*	**77.0**	*0.80*	**77.9**	*0.86*

	78.9	*1.56*	**78.6**	*0.98*	**78.0**	*1.87*	**79.0**	*1.34*	**78.3**	*1.53*	**78.4**	*1.29*
EXTEM CT (sec)	**72**	*16.09*	**70**	*17.80*	**116**	*43.05*	**56**	*5.07*	**67**	*5.25*	**77**	*8.30*
	63	*5.66*	**110**	*9.78*	**71**	*11.53*	**70**	*7.03*	**84**	*12.27*	**76**	*8.68*
	58	*5.59*	**67**	*7.72*	**75**	*9.37*	**58**	*5.49*	**63**	*6.78*	**63**	*4.40*
	74	*4.51*	**79**	*4.95*	**72**	*4.87*	**72**	*3.48*	**75**	*5.56*	**73**	*5.90*
EXTEM CFT (sec)	**82**	*8.97*	**107**	*6.91*	**103**	*8.32*	**83**	*7.16*	**73**	*6.43*	**75**	*8.54*
	69	*4.16*	**101**	*6.87*	**76**	*5.43*	**68**	*5.80*	**71**	*8.24*	**94**	*13.33*
	80	*12.57*	**87**	*15.86*	**104**	*8.70*	**81**	*8.38*	**89**	*7.06*	**96**	*7.05*
	69	*4.96*	**82**	*13.33*	**94**	*19.62*	**80**	*7.62*	**81**	*10.10*	**88**	*6.85*
EXTEM MCF (sec)	**75.6**	*2.63*	**77.1**	*0.95*	**77.8**	*1.03*	**79.1**	*1.16*	**78.3**	*1.00*	**79.9**	*1.13*
	81.8	*1.05*	**76.9**	*2.09*	**79.9**	*1.80*	**80.4**	*0.92*	**81.1**	*1.44*	**77.1**	*1.87*
	80.1	*2.17*	**80.0**	*1.86*	**80.4**	*1.36*	**80.3**	*1.18*	**75.9**	*2.19*	**76.0**	*2.36*
	82.5	*1.16*	**80.9**	*1.76*	**78.5**	*2.33*	**80.5**	*1.68*	**77.8**	*2.45*	**79.0**	*1.95*
EXTEM Angle	**77.4**	*1.81*	**73.4**	*1.25*	**75.1**	*1.42*	**79.1**	*0.97*	**78.8**	*0.98*	**78.1**	*1.67*
	78.0	*0.85*	**72.4**	*1.13*	**76.9**	*1.30*	**79.0**	*0.98*	**78.6**	*1.25*	**73.6**	*2.56*
	78.8	*1.25*	**73.0**	*2.85*	**73.8**	*1.96*	**76.6**	*1.49*	**76.5**	*1.05*	**73.0**	*1.88*
	79.5	*0.73*	**77.9**	*2.04*	**75.8**	*2.25*	**77.4**	*1.41*	**79.0**	*0.96*	**77.1**	*0.74*
Platelet Count	**482**	*42.29*	**316**	*64.70*	**454**	*39.74*	**425**	*40.55*	**407**	*50.17*	**439**	*58.63*
	628	*85.22*	**440**	*50.52*	**539**	*85.52*	**480**	*102.84*	**459**	*87.70*	**420**	*84.51*
	623	*33.99*	**400**	*327.67*	**474**	*60.96*	**463**	*38.87*	**437**	*33.63*	**428**	*53.15*
	471	*54.39*	**758**	*81.78*	**438**	*40.12*	**482**	*80.38*	**436**	*89.42*	**445**	*64.90*
Fibrinogen	**203**	*19.26*	**190**	*21.58*	**196**	*19.53*	**248**	*24.52*	**291**	*79.36*	**262**	*53.62*
	373	*37.08*	**253**	*36.33*	**329**	*31.58*	**272**	*37.70*	**246**	*25.95*	**223**	*26.89*
	249	*24.33*	**218**	*39.53*	**229**	*56.35*	**234**	*32.04*	**218**	*20.59*	**209**	*24.66*
	232	*57.95*	**256**	*57.13*	**214**	*71.69*	**253**	*51.24*	**197**	*40.34*	**190**	*48.47*
COLL/EPI PFA	**268.6**	*20.98*	**210.0**	*18.83*	**207.3**	*33.41*	**180.1**	*27.27*	**229.9**	*34.49*	**235.9**	*32.48*
	283.5	*16.50*	**256.6**	*28.83*	**262.3**	*25.31*	**242.1**	*27.51*	**217.8**	*32.15*	**225.8**	*30.62*
	264.0	*23.87*	**274.3**	*17.46*	**262.8**	*19.36*	**234.4**	*25.65*	**260.3**	*21.05*	**266.5**	*24.35*
	163.5	*9.98*	**208.1**	*28.88*	**235.5**	*47.95*	**238.3**	*26.43*	**269.3**	*20.59*	**222.9**	*24.99*
COLL/ADP PFA	**134.4**	*36.75*	**147.4**	*34.05*	**170.6**	*46.23*	**104.3**	*14.35*	**155.0**	*37.83*	**118.4**	*29.09*
	88.0	*9.77*	**89.1**	*8.62*	**74.8**	*7.09*	**94.1**	*11.27*	**100.8**	*16.41*	**104.0**	*13.86*
	167.6	*39.80*	**88.3**	*10.96*	**109.6**	*28.08*	**143.8**	*32.81*	**85.7**	*8.15*	**81.7**	*9.03*
	77.9	*8.48*	**112.8**	*27.17*	**94.8**	*15.45*	**89.9**	*9.80*	**177.5**	*37.13*	**109.5**	*28.28*
	9.5	*2.12*	**8.1**	*0.81*	**9.5**	*1.96*	**10.0**	*1.72*	**8.5**	*2.33*	**11.6**	*2.34*

FOT (min)	**6.8**	*1.44*	**15.9**	*1.61*	**7.6**	*1.65*	**8.9**	*1.37*	**9.4**	*1.22*	**8.5**	*1.70*
	10.0	*2.62*	**8.7**	*2.68*	**9.2**	*2.27*	**8.3**	*2.70*	**13.3**	*2.17*	**8.0**	*1.45*
	13.9	*1.86*	**12.4**	*1.84*	**12.8**	*3.90*	**15.0**	*1.69*	**15.5**	*2.35*	**11.3**	*2.20*
CEM (kdynes/cm2)	**29.0**	*8.68*	**20.7**	*2.44*	**20.1**	*4.88*	**27.3**	*10.69*	**36.3**	*11.73*	**25.9**	*8.22*
	56.3	*11.84*	**0.9**	*0.56*	**18.3**	*4.40*	**36.3**	*6.14*	**33.9**	*5.61*	**42.2**	*12.76*
	33.3	*12.15*	**42.0**	*14.38*	**35.4**	*12.32*	**39.5**	*14.49*	**18.0**	*5.17*	**42.3**	*16.12*
	20.6	*8.78*	**29.8**	*11.60*	**23.1**	*14.58*	**16.4**	*5.56*	**10.2**	*5.78*	**24.3**	*9.69*
PCF (kdynes)	**7.9**	*2.73*	**7.1**	*0.89*	**7.6**	*2.35*	**6.6**	*2.31*	**10.6**	*3.19*	**6.2**	*2.05*
	13.4	*3.87*	**0.6**	*0.26*	**7.9**	*2.18*	**8.1**	*2.05*	**6.1**	*1.57*	**10.3**	*3.59*
	9.2	*3.25*	**11.3**	*3.94*	**9.7**	*2.77*	**9.3**	*3.59*	**3.9**	*1.01*	**11.0**	*4.27*
	6.0	*2.63*	**6.1**	*2.44*	**5.3**	*3.57*	**2.8**	*1.00*	**2.6**	*1.42*	**6.8**	*3.00*
Liver Enzymes	**15**	*0.87*	**16**	*0.80*	**21**	*2.68*	**36**	*5.14*	**32**	*4.29*	**21**	*2.18*
	15	*0.94*	**13**	*1.88*	**17**	*2.25*	**25**	*2.57*	**25**	*4.17*	**20**	*3.65*
	13	*1.42*	**12**	*1.32*	**15**	*2.53*	**21**	*3.17*	**18**	*2.75*	**17**	*1.82*
	14	*1.47*	**10**	*1.17*	**17**	*0.92*	**25**	*3.56*	**27**	*5.44*	**22**	*3.28*
Platelets CD62p	**19.88**	*5.03*	**15.18**	*3.97*	**9.91**	*1.75*	**15.91**	*3.69*	**20.11**	*3.91*	**15.04**	*3.04*
	33.95	*3.87*	**16.04**	*1.45*	**26.83**	*4.17*	**23.99**	*3.93*	**27.09**	*4.11*	**32.20**	*6.38*
	19.82	*7.28*	**17.28**	*6.73*	**12.60**	*2.66*	**12.01**	*3.54*	**14.11**	*1.98*	**17.50**	*5.59*
	14.42	*2.43*	**15.40**	*3.12*	**8.58**	*2.49*	**16.75**	*6.28*	**11.72**	*2.92*	**13.45**	*4.57*
vWF:Ag	**88.27**	*15.17*	**89.69**	*13.02*	**94.58**	*15.66*	**97.06**	*20.43*	**83.74**	*17.52*	**70.11**	*13.16*
	82	*4.6*	**80.5**	*6.95*	**94.13**	*12.95*	**98.38**	*5.96*	**106.88**	*11.9*	**89.38**	*7.59*
	93.44	*16.89*	**79.43**	*13.91*	**86.41**	*15.15*	**66.17**	*11.45*	**72.44**	*13.8*	**76.4**	*14.39*
	76.13	*6.83*	**91**	*5.03*	**82.75**	*7.16*	**80.5**	*5.33*	**89.38**	*8.83*	**79.25**	*6.2*
Collagen Agg (ohms)	**9.8**	*1.53*	**11.4**	*1.11*	**11.2**	*1.45*	**12.1**	*1.57*	**11.1**	*1.27*	**11.6**	*2.34*
	9.0	*0.91*	**10.1**	*1.00*	**12.3**	*1.07*	**10.3**	*1.29*	**8.8**	*1.18*	**10.0**	*0.53*
	11.3	*1.73*	**11.5**	*0.78*	**10.4**	*1.03*	**9.4**	*1.53*	**12.0**	*1.05*	**10.9**	*0.97*
	12.6	*0.70*	**10.2**	*1.38*	**8.4**	*0.85*	**10.1**	*0.88*	**10.8**	*1.93*	**10.4**	*0.88*
ADP Agg (Ohms)	**9.6**	*1.19*	**10.7**	*0.86*	**10.6**	*1.16*	**10.4**	*1.11*	**10.3**	*1.04*	**10.7**	*1.16*
	9.1	*0.56*	**9.6**	*0.66*	**11.3**	*0.92*	**10.6**	*1.14*	**8.8**	*0.75*	**9.8**	*0.89*
	10.8	*0.63*	**10.6**	*0.66*	**10.3**	*1.00*	**8.9**	*1.45*	**9.7**	*0.68*	**10.6**	*0.80*
	12.3	*0.98*	**28.3**	*19.34*	**9.3**	*1.16*	**9.0**	*0.96*	**9.9**	*1.25*	**9.7**	*0.95*
CAT Lag	**4.9**	*0.60*	**5.4**	*0.75*	**4.4**	*0.43*	**5.4**	*0.59*	**4.2**	*0.59*	**5.2**	*1.13*
	5.4	*0.51*	**7.4**	*1.26*	**5.3**	*0.54*	**4.7**	*0.34*	**5.4**	*0.49*	**5.3**	*1.12*
	4.9	*0.49*	**4.7**	*0.54*	**4.9**	*0.72*	**4.7**	*0.67*	**4.0**	*0.30*	**4.8**	*0.42*

	5.8	*0.46*	**4.8**	*0.49*	**6.1**	*0.62*	**5.0**	*0.26*	**4.6**	*0.30*	**4.7**	*0.48*
CAT ETP	**950.6**	*159.64*	**855.4**	*174.27*	**836.2**	*186.09*	**849.1**	*179.42*	**752.6**	*150.67*	**883.5**	*146.48*
	864.3	*172.39*	**1099.4**	*205.84*	**1087.6**	*189.58*	**852.4**	*250.29*	**786.3**	*103.65*	**809.9**	*243.45*
	955.2	*105.56*	**1077.7**	*149.62*	**1046.1**	*153.48*	**904.5**	*94.02*	**701.6**	*123.59*	**860.8**	*195.44*
	1091.4	*142.56*	**1000.1**	*200.58*	**1452.1**	*244.81*	**1065.9**	*156.70*	**767.0**	*103.80*	**1015.5**	*229.08*
CAT Peak	**53.7**	*10.43*	**52.7**	*16.53*	**58.0**	*15.89*	**58.3**	*17.72*	**51.4**	*14.69*	**52.0**	*7.35*
	47.2	*15.91*	**61.7**	*16.86*	**104.0**	*29.19*	**49.5**	*21.97*	**34.6**	*5.33*	**47.8**	*17.47*
	62.3	*11.52*	**57.9**	*9.93*	**65.8**	*9.49*	**52.4**	*7.54*	**41.0**	*9.22*	**46.1**	*10.11*
	67.5	*13.95*	**49.8**	*8.46*	**87.3**	*19.00*	**62.6**	*13.61*	**45.4**	*10.77*	**50.5**	*12.05*
CAT Tip	**16.3**	*1.25*	**17.0**	*1.73*	**13.9**	*1.26*	**15.3**	*1.10*	**16.0**	*1.94*	**16.7**	*1.27*
	19.3	*2.31*	**23.2**	*3.15*	**15.6**	*0.94*	**16.6**	*1.65*	**16.6**	*1.83*	**16.0**	*1.38*
	17.8	*1.92*	**18.4**	*1.79*	**15.7**	*1.31*	**16.3**	*1.51*	**15.9**	*1.72*	**17.1**	*0.75*
	19.8	*1.38*	**17.7**	*1.11*	**18.1**	*2.72*	**18.2**	*1.41*	**18.5**	*1.61*	**20.3**	1.71
Neutrophilis	**139.50**	*5.71*	**168.57**	*35.43*	**138.17**	*23.83*	**184.00**	*28.29*	**132.80**	*25.25*	**130.00**	*27.31*
	112.00	*13.25*	**54.86**	*15.17*	**88.50**	*7.38*	**102.00**	*27.93*	**98.80**	*7.79*	**97.29**	*13.12*
	154.00	*33.41*	**134.29**	*15.23*	**185.50**	*28.35*	**149.00**	*33.99*	**157.75**	*12.34*	**207.00**	
	102.88	*18.03*	**119.00**	*16.69*	**107.75**	*9.32*	**131.00**	*19.24*	**161.38**	*18.11*	**90.50**	*12.26*
Monocytes	**134.67**	*20.68*	**171.57**	*37.04*	**150.00**	*21.63*	**165.20**	*31.57*	**159.00**	*41.04*	**186.20**	*35.85*
	172.14	*28.61*	**171.86**	*23.82*	**190.50**	*34.37*	**194.40**	*62.24*	**192.75**	*27.27*	**179.67**	*55.86*
	108.25	*20.81*	**106.63**	*13.57*	**71.50**	*5.01*	**130.88**	*24.15*	**130.50**	*14.63*	**94.40**	*14.93*
	129.76	*10.01*	**75.97**	*13.58*	**102.26**	*8.85*	**96.28**	*5.87*	**114.14**	*6.95*	**107.97**	*11.44*

Notes:

	Oxygent
	PeftToran
	Hetastarch
	Control

Table 3: Summary data of Task2

Parameter	Baseline		1 Hour		24 Hour		96 hour		7 Days	
Hemorrhage	**Mean**	*SE*	**Mean**	*SE*	**Mean**	*SE*	**Mean**	*SE*	**Mean**	*SE*
NATEM CT (sec)	**546.33**	*94.66*	**471.78**	*38.54*	**670.00**	*96.53*	**682.11**	*102.46*	**663.33**	*103.93*
	479.60	*43.20*	**562.30**	*46.78*	**537.20**	*50.12*	**400.90**	*35.21*	**422.40**	*37.07*
	550.70	*48.64*	**539.30**	*30.73*	**566.60**	*73.67*	**552.10**	*34.37*	**508.80**	*52.48*
	487.50	*45.80*	**562.30**	*41.58*	**537.20**	*58.42*	**400.90**	*59.32*	**422.40**	*48.36*
NATEM CFT (sec)	**170.56**	*28.14*	**169.22**	*11.64*	**186.56**	*19.40*	**195.67**	*33.66*	**178.22**	*45.08*
	144.90	*21.72*	**193.20**	*19.27*	**153.30**	*10.64*	**113.30**	*11.52*	**104.10**	*10.20*
	168.90	*20.43*	**157.70**	*17.19*	**166.80**	*25.17*	**154.80**	*15.94*	**141.10**	*15.05*
	142.70	*16.86*	**193.20**	*13.66*	**153.30**	*19.05*	**113.30**	*16.53*	**104.10**	*16.80*
NATEM MCF (mm)	**72.44**	*1.71*	**64.67**	*1.88*	**76.33**	*1.33*	**79.56**	*1.23*	**81.11**	*1.73*
	75.40	*1.78*	**65.20**	*1.75*	**74.10**	*1.89*	**78.30**	*2.24*	**76.40**	*1.43*
	73.10	*1.89*	**73.60**	*2.25*	**76.30**	*1.89*	**76.70**	*1.32*	**76.90**	*1.67*
	73.90	*1.43*	**65.20**	*1.19*	**74.10**	*1.26*	**78.30**	*1.04*	**76.40**	*1.92*
NATEM Angle (%)	**61.67**	*3.48*	**58.78**	*1.74*	**59.00**	*2.57*	**61.00**	*3.82*	**64.56**	*4.46*
	65.20	*2.89*	**56.60**	*2.66*	**63.10**	*1.67*	**70.30**	*2.01*	**70.90**	*1.72*
	61.70	*3.03*	**62.80**	*2.30*	**62.20**	*2.96*	**63.90**	*2.38*	**65.80**	*2.11*
	64.40	*2.64*	**56.60**	*1.99*	**63.10**	*2.76*	**70.30**	*2.34*	**70.90**	*2.63*
INTEM CT(sec)	**159.78**	*12.61*	**187.33**	*14.39*	**177.78**	*12.08*	**156.89**	*13.25*	**171.89**	*13.94*
	144.60	*7.07*	**193.70**	*6.56*	**151.40**	*5.74*	**139.20**	*5.94*	**155.90**	*5.16*
	157.60	*8.55*	**156.10**	*8.83*	**163.30**	*7.78*	**159.80**	*4.23*	**149.30**	*9.81*
	156.10	*7.95*	**193.70**	*8.60*	**151.40**	*11.34*	**139.20**	*6.82*	**155.90**	*9.44*
INTEM CFT (sec)	**72.00**	*4.57*	**155.89**	*16.29*	**78.44**	*10.40*	**48.67**	*3.13*	**44.89**	*2.82*
	56.40	*4.53*	**148.60**	*18.47*	**63.20**	*7.18*	**49.50**	*6.86*	**45.60**	*3.58*
	67.30	*8.57*	**67.80**	*22.36*	**51.40**	*9.03*	**54.00**	*2.84*	**48.90**	*2.00*
	53.10	*3.08*	**148.60**	*7.10*	**63.20**	*3.00*	**49.50**	*3.68*	**45.60**	*3.01*
INTEM MCF (mm)	**75.89**	*1.22*	**136.67**	*70.06*	**152.44**	*74.95*	**81.67**	*0.93*	**83.44**	*1.36*
	78.70	*1.28*	**67.00**	*1.91*	**76.90**	*1.58*	**78.50**	*2.00*	**79.10**	*1.59*
	77.20	*1.55*	**76.50**	*2.33*	**79.90**	*1.41*	**80.20**	*1.28*	**80.20**	*1.24*
	78.40	*0.88*	**67.00**	*1.35*	**76.90**	*1.02*	**78.50**	*1.31*	**79.10**	*1.55*
INTEM Angle (%)	**76.44**	*0.82*	**61.22**	*2.46*	**77.33**	*1.04*	**80.22**	*0.55*	**80.89**	*0.39*
	78.60	*0.83*	**62.80**	*2.84*	**78.50**	*0.86*	**80.70**	*0.76*	**81.30**	*0.56*
	76.90	*1.45*	**76.40**	*2.85*	**79.60**	*0.95*	**79.30**	*0.61*	**79.90**	*0.45*

	79.20	*0.57*	**62.80**	*1.44*	**78.50**	*0.58*	**80.70**	*0.62*	**81.30**	*0.64*
EXTEM CT (sec)	**74.44**	*5.83*	**94.00**	*10.87*	**68.78**	*5.58*	**78.22**	*9.71*	**78.11**	*8.33*
	80.30	*3.05*	**124.20**	*5.25*	**76.90**	*2.69*	**79.40**	*3.91*	**81.30**	*2.74*
	72.30	*6.06*	**66.10**	*4.80*	**71.40**	*5.46*	**83.30**	*9.06*	**79.80**	*8.28*
	62.80	*6.22*	**124.20**	*6.99*	**76.90**	*7.09*	**79.40**	*10.71*	**81.30**	*9.57*
EXTEM CFT (sec)	**96.11**	*5.87*	**224.00**	*17.88*	**104.22**	*18.48*	**52.56**	*5.08*	**57.44**	*5.01*
	76.80	*5.49*	**221.90**	*20.92*	**71.40**	*8.26*	**57.60**	*7.17*	**64.00**	*4.01*
	88.20	*9.22*	**104.60**	*20.63*	**67.80**	*9.73*	**71.50**	*5.78*	**73.20**	*21.46*
	84.90	*6.69*	**221.90**	*6.40*	**71.40**	*5.20*	**57.60**	*4.69*	**64.00**	*5.91*
EXTEM MCF (sec)	**75.89**	*1.21*	**66.78**	*2.01*	**76.33**	*1.27*	**82.11**	*0.95*	**83.22**	*1.52*
	78.60	*1.55*	**69.20**	*1.96*	**77.50**	*1.61*	**78.90**	*2.21*	**79.50**	*1.61*
	77.30	*1.58*	**76.40**	*2.03*	**79.80**	*2.75*	**80.10**	*1.34*	**80.50**	*1.39*
	78.10	*0.84*	**69.20**	*1.17*	**77.50**	*0.87*	**78.90**	*1.39*	**79.50**	*1.81*
EXTEM Angle	**74.44**	*1.72*	**54.56**	*3.19*	**77.89**	*2.08*	**81.44**	*0.50*	**160.33**	*81.46*
	78.20	*1.22*	**55.90**	*3.37*	**79.70**	*0.88*	**80.90**	*0.69*	**79.30**	*0.86*
	75.60	*1.77*	**74.50**	*3.69*	**79.40**	*0.80*	**78.50**	*0.90*	**77.40**	*0.87*
	75.90	*1.28*	**55.90**	*1.29*	**79.70**	*0.76*	**80.90**	*0.83*	**79.30**	*1.28*
Platelet Count	**533.89**	*67.55*	**271.67**	*61.50*	**271.67**	*54.11*	**340.78**	*51.09*	**460.44**	*26.69*
	447.47	*38.76*	**153.48**	*20.87*	**188.47**	*18.14*	**355.45**	*62.11*	**447.27**	*30.99*
	498.70	*38.56*	**336.40**	*41.87*	**484.90**	*35.46*	**497.10**	*44.30*	**528.50**	*78.82*
	562.00	*60.10*	**153.48**	*48.70*	**188.47**	*56.65*	**355.45**	*49.42*	**447.27**	*39.94*
Fibrinogen	**172.22**	*10.28*	**109.22**	*7.95*	**225.78**	*17.20*	**311.00**	*29.86*	**260.56**	*30.12*
	226.20	*21.29*	**127.00**	*11.87*	**220.30**	*25.64*	**237.50**	*27.61*	**192.80**	*13.41*
	191.70	*19.25*	**170.20**	*15.19*	**214.10**	*16.56*	**259.30**	*39.38*	**237.40**	*44.63*
	176.80	*9.19*	**127.00**	*13.66*	**220.30**	*17.94*	**237.50**	*43.95*	**192.80**	*47.59*
Coll/Epi PFA	**247.22**	*21.54*	**300.00**	*0.00*	**270.44**	*15.27*	**223.22**	*24.92*	**222.11**	*20.98*
	217.10	*24.94*	**291.00**	*9.00*	**239.40**	*23.46*	**184.40**	*24.34*	**225.00**	*26.58*
	218.30	*25.08*	**265.80**	*13.20*	**198.10**	*18.15*	**213.80**	*16.45*	**144.60**	*21.12*
	227.50	*26.87*	**291.00**	*18.03*	**239.40**	*30.14*	**184.40**	*25.86*	**225.00**	*9.12*
Coll/ADP PFA	**84.44**	*7.00*	**124.33**	*26.03*	**193.22**	*34.60*	**85.56**	*12.53*	**85.44**	*6.81*
	106.50	*21.97*	**171.60**	*28.21*	**114.50**	*14.17*	**127.00**	*26.08*	**78.60**	*7.76*
	143.60	*27.25*	**71.10**	*28.12*	**82.80**	*22.01*	**107.70**	*15.30*	**75.90**	*6.13*
	74.40	*6.37*	**171.60**	*5.24*	**114.50**	*9.14*	**127.00**	*25.37*	**78.60**	*8.28*
FOT (min)	**9.22**	*2.20*	**8.33**	*1.00*	**11.22**	*1.81*	**11.67**	*2.33*	**10.33**	*1.94*
	7.90	*0.99*	**10.70**	*1.59*	**8.40**	*0.83*	**5.90**	*0.71*	**6.00**	*0.68*

	8.90	*0.90*	**8.60**	*0.96*	**8.10**	*1.54*	**8.80**	*0.91*	**7.30**	*1.34*
	7.80	*1.02*	**10.70**	*0.97*	**8.40**	*1.11*	**5.90**	*1.24*	**6.00**	*0.98*
CEM (kdynes/cm2)	**37.95**	*8.80*	**17.06**	*2.69*	**25.21**	*7.57*	**31.60**	*9.11*	**39.38**	*8.84*
	51.46	*7.55*	**2.55**	*0.62*	**47.72**	*6.40*	**70.38**	*7.97*	**57.01**	*7.62*
	33.11	*5.07*	**38.12**	*4.78*	**48.63**	*8.63*	**39.36**	*5.77*	**54.88**	*11.28*
	38.69	*3.38*	**2.55**	*5.46*	**47.72**	*6.30*	**70.38**	*5.99*	**57.01**	*6.45*
PCF (kdynes)	**8.51**	*2.20*	**4.54**	*1.06*	**5.82**	*1.77*	**7.21**	*2.19*	**11.09**	*2.92*
	9.66	*2.08*	**1.66**	*0.30*	**8.42**	*1.50*	**13.78**	*2.21*	**12.32**	*1.83*
	6.46	*1.43*	**8.06**	*1.05*	**10.93**	*2.50*	**8.31**	*1.93*	**12.51**	*3.44*
	9.64	*1.76*	**1.66**	*1.55*	**8.42**	*1.99*	**13.78**	*1.66*	**12.32**	*2.22*
Liver Enzyme	**11.11**	*1.64*	**10.22**	*1.40*	**33.78**	*7.54*	**31.33**	*7.21*	**19.00**	*3.76*
	18.70	*1.33*	**12.89**	*1.07*	**29.22**	*4.10*	**28.20**	*3.33*	**20.80**	*2.42*
	17.40	*1.08*	**15.50**	*1.29*	**33.60**	*4.96*	**40.70**	*3.54*	**25.33**	*3.36*
	18.50	*1.46*	**12.89**	*0.73*	**29.22**	*3.53*	**28.20**	*5.66*	**20.80**	*2.67*
Platelets CD62p	**25.83**	*4.92*	**17.44**	*2.76*	**18.81**	*3.90*	**25.71**	*7.83*	**30.27**	*8.63*
	24.04	*3.29*	**23.26**	*2.66*	**25.55**	*2.38*	**26.87**	*2.19*	**28.03**	*2.74*
	27.17	*4.58*	**16.10**	*3.68*	**24.44**	*3.38*	**34.29**	*5.19*	**31.90**	*3.86*
	30.39	*3.67*	**23.26**	*2.89*	**25.55**	*4.11*	**26.87**	*5.14*	**28.03**	*4.08*
vWF: Ag	**63.42**	*8.80*	**57.78**	*7.46*	**72.35**	*12.17*	**77.35**	*10.61*	**72.56**	*9.46*
	87.20	*6.29*	**65.70**	*5.39*	**88.00**	*6.04*	**99.70**	*8.41*	**103.10**	*7.02*
	75.30	*3.85*	**68.60**	*4.33*	**69.20**	*3.92*	**83.20**	*6.92*	**76.10**	*6.10*
	75.90	*4.69*	**65.70**	*4.43*	**88.00**	*5.65*	**99.70**	*7.92*	**103.10**	*3.10*
Collagen Agg (Ohms)	**8.72**	*0.53*	**4.39**	*1.02*	**5.56**	*0.78*	**8.33**	*0.46*	**10.39**	*0.90*
	7.95	*0.59*	**3.78**	*0.83*	**6.06**	*1.13*	**8.50**	*1.00*	**10.50**	*0.89*
	9.22	*0.70*	**9.43**	*0.82*	**9.56**	*0.82*	**8.50**	*0.77*	**10.31**	*0.78*
	9.39	*0.56*	**3.78**	*0.69*	**6.06**	*1.04*	**8.50**	*0.84*	**10.50**	*0.86*
ADP Agg (Ohms)	**7.94**	*0.71*	**6.00**	*0.75*	**7.00**	*1.30*	**9.61**	*0.74*	**9.50**	*0.60*
	9.30	*1.02*	**6.85**	*0.67*	**9.00**	*0.96*	**10.35**	*1.04*	**11.64**	*1.04*
	10.28	*0.77*	**9.39**	*0.63*	**10.17**	*0.75*	**8.89**	*0.71*	**11.00**	*0.86*
	8.83	*1.33*	**6.85**	*0.64*	**9.00**	*1.26*	**10.35**	*0.36*	**11.64**	*0.89*
CAT lag	**4.78**	*0.84*	**5.32**	*0.40*	**6.18**	*0.26*	**5.80**	*0.83*	**6.61**	*0.26*
	7.38	*0.68*	**8.51**	*0.76*	**6.83**	*0.25*	**7.95**	*0.73*	**7.45**	*0.61*
	6.22	*0.46*	**5.22**	*0.26*	**6.80**	*0.47*	**6.62**	*1.25*	**6.62**	*0.79*
	4.99	*0.19*	**8.51**	*0.40*	**6.83**	*0.85*	**7.95**	*0.40*	**7.45**	*0.98*
	946.63	*265.29*	**767.97**	*106.86*	**806.00**	*147.99*	**1496.88**	*228.75*	**1261.41**	*206.01*

CAT ETP	**1066.86**	*126.38*	**732.11**	*137.55*	**1032.55**	*128.97*	**1404.96**	*152.87*	**1357.70**	*159.44*
	1283.80	*211.84*	**1161.50**	*139.60*	**1220.92**	*168.03*	**1338.69**	*120.54*	**1453.35**	*185.26*
	1141.66	*106.75*	**732.11**	*100.08*	**1032.55**	*95.53*	**1404.96**	*44.68*	**1357.70**	*139.30*
CAT Peak	**48.09**	*17.04*	**42.71**	*5.00*	**40.95**	*8.12*	**85.86**	*17.90*	**75.13**	*14.79*
	61.06	*9.33*	**38.16**	*6.65*	**52.25**	*6.71*	**88.98**	*12.19*	**97.28**	*14.64*
	66.07	*14.36*	**65.32**	*61.54*	**62.23**	*7.40*	**79.69**	*10.82*	**86.95**	*15.28*
	74.67	*9.31*	**38.16**	*9.75*	**52.25**	*6.63*	**88.98**	*7.88*	**97.28**	*10.97*
CAT Tip	**16.24**	*1.47*	**15.09**	*1.81*	**16.72**	*0.94*	**18.53**	*1.84*	**19.09**	*1.39*
	19.49	*1.11*	**20.93**	*1.34*	**19.80**	*0.78*	**17.85**	*1.03*	**16.54**	*0.72*
	18.97	*1.02*	**16.86**	*0.84*	**19.40**	*1.22*	**18.58**	*1.38*	**17.66**	*1.95*
	15.62	*0.92*	**20.93**	*0.92*	**19.80**	*0.81*	**17.85**	*1.14*	**16.54**	*1.15*
Neutrophils	**179.3463**	*30.24*	**157.3663**	*20.23*	**170.2975**	*23.90*	**136.11**	*14.63*	**149.17**	*26.64*
	510.375	*114.60*	**466.25**	*78.79*	**393.5714**	*71.44*	**521.8571**	*85.56*	**575.7143**	*82.94*
	111.0311	*10.19*	**105.196**	*8.30*	**146.526**	*27.75*	**146.502**	*38.78*	**141.57**	*31.75*
	107.406	*15.03*	**466.25**	*15.37*	**393.5714**	*16.90*	**521.8571**	*27.16*	**575.7143**	*22.59*
Monocytes	**101.59**	*4.33*	**108.66**	*8.30*	**84.83**	*4.48*	**1289.98**	*1200.02*	**98.20**	*7.27*
	289.67	*23.48*	**211.40**	*48.35*	**259.13**	*21.92*	**327.86**	*19.06*	**274.67**	*34.13*
	106.56	*11.90*	**90.31**	*6.57*	**86.17**	*21.07*	**90.06**	*23.92*	**100.97**	*28.88*
	93.17	*4.31*	**211.40**	*3.90*	**259.13**	*3.91*	**327.86**	*3.38*	**274.67**	*3.33*
Natem TPI	**62.06**	*13.82*	**36.51**	*5.67*	**61.22**	*10.04*	**82.75**	*16.34*	**115.85**	*23.85*
	87.84	*19.07*	**35.61**	*6.89*	**63.94**	*8.32*	**119.00**	*18.68*	**109.68**	*16.95*
	64.26	*14.73*	**59.03**	*6.04*	**72.52**	*14.29*	**73.53**	*23.14*	**93.91**	*26.53*
	73.43	*11.93*	**35.61**	*6.43*	**63.94**	*13.46*	**119.00**	*9.29*	**109.68**	*17.36*
Intem TPI	**141.55**	*15.77*	**45.89**	*7.39*	**153.08**	*21.42*	**295.43**	*31.52*	**365.77**	*36.14*
	224.20	*30.21*	**51.97**	*9.22*	**209.47**	*50.02*	**279.61**	*39.44*	**290.87**	*41.86*
	195.86	*44.28*	**170.87**	*22.82*	**252.12**	*32.95*	**256.10**	*56.89*	**296.59**	*43.42*
	220.43	*23.22*	**51.97**	*24.89*	**209.47**	*28.87*	**279.61**	*37.22*	**290.87**	*55.00*
Extem TPI	**105.01**	*10.80*	**31.19**	*5.23*	**120.65**	*20.72*	**300.77**	*42.76*	**309.24**	*47.21*
	168.26	*26.52*	**36.76**	*6.31*	**196.58**	*48.28*	**260.94**	*49.88*	**209.13**	*30.71*
	160.95	*49.45*	**100.98**	*14.86*	**192.90**	*40.13*	**192.04**	*51.57*	**233.02**	*59.27*
	138.16	*15.35*	**36.76**	*11.29*	**196.58**	*23.92*	**260.94**	*26.03*	**209.13**	*62.15*

	Not enough data
	Oxygent
	Perftoran
	Saline
	Control

Table 4: Summary data of Task3

Parameter	Baseline		1 Hour		24 Hour		96 hour		7 Days	
Poly	**Mean**	*SE*	**Mean**	*SE*	**Mean**	*SE*	**Mean**	*SE*	**Mean**	*SE*
NATEM CT (sec)										
	463.10	30.60	532.40	23.92	540.90	81.00	632.50	82.27	532.20	60.73
	473.00	78.67	451.90	36.30	522.90	52.71	445.30	73.56	386.60	43.89
	487.50	*45.80*	**562.30**	*41.58*	**537.20**	*58.42*	**400.90**	*59.32*	**422.40**	*48.36*
NATEM CFT (sec)										
	123.30	11.94	190.20	10.40	175.70	18.91	177.50	25.10	131.05	16.77
	147.40	22.25	167.20	14.11	151.90	19.41	125.10	19.60	96.30	15.22
	142.70	*16.86*	**193.20**	*13.66*	**153.30**	*19.05*	**113.30**	*16.53*	**104.10**	*16.80*
NATEM MCF (mm)										
	75.90	1.46	63.00	1.69	76.50	1.18	77.70	2.48	78.10	1.85
	73.20	2.17	63.90	2.96	75.80	1.51	82.40	1.32	81.60	1.85
	73.90	*1.43*	**65.20**	*1.19*	**74.10**	*1.26*	**78.30**	*1.04*	**76.40**	*1.92*
NATEM Angle (%)										
	67.80	2.02	56.50	1.57	63.40	3.07	63.50	2.92	68.80	2.29
	64.50	3.09	59.50	2.25	64.20	2.84	69.90	2.61	74.20	2.30
	64.40	*2.64*	**56.60**	*1.99*	**63.10**	*2.76*	**70.30**	*2.34*	**70.90**	*2.63*
INTEM CT(sec)										
	160.00	5.86	204.00	11.24	168.10	11.66	178.80	18.59	164.90	8.99
	168.00	10.37	181.90	11.69	156.30	6.30	137.40	10.82	148.40	6.98
	156.10	*7.95*	**193.70**	*8.60*	**151.40**	*11.34*	**139.20**	*6.82*	**155.90**	*9.44*
INTEM CFT (sec)										
	60.30	4.14	163.80	20.42	67.80	5.27	48.40	5.18	42.70	2.47

	65.90	6.65	153.10	17.70	64.80	5.30	46.70	3.79	44.00	0.82
	53.10	*3.08*	**148.60**	*7.10*	**63.20**	*3.00*	**49.50**	*3.68*	**45.60**	*3.01*
INTEM MCF (mm)										
	77.90	1.11	66.20	1.58	76.30	0.75	82.30	1.11	82.30	1.62
	74.80	1.87	65.50	2.39	75.60	1.24	81.50	0.98	82.10	1.11
	78.40	*0.88*	**67.00**	*1.35*	**76.90**	*1.02*	**78.50**	*1.31*	**79.10**	*1.55*
INTEM Angle (%)										
	77.90	0.87	60.60	3.10	77.50	0.98	80.10	0.87	81.70	0.50
	77.10	1.29	62.60	2.62	78.50	0.75	80.90	0.46	81.20	0.25
	79.20	*0.57*	**62.80**	*1.44*	**78.50**	*0.58*	**80.70**	*0.62*	**81.30**	*0.64*
EXTEM CT (sec)										
	92.80	12.81	174.00	23.61	87.00	7.36	87.70	7.44	81.80	4.72
	85.20	7.02	137.90	31.00	93.20	13.37	91.90	9.66	93.10	14.24
	62.80	*6.22*	**124.20**	*6.99*	**76.90**	*7.09*	**79.40**	*10.71*	**81.30**	*9.57*
EXTEM CFT (sec)										
	90.90	8.37	206.90	8.24	89.70	7.90	52.70	6.25	54.30	5.26
	103.40	12.06	235.70	28.29	88.50	11.81	59.50	8.36	77.20	7.68
	84.90	*6.69*	**221.90**	*6.40*	**71.40**	*5.20*	**57.60**	*4.69*	**64.00**	*5.91*
EXTEM MCF (sec)										
	78.78	1.19	66.22	2.10	76.56	0.77	83.33	1.39	82.22	1.84
	74.40	1.93	66.10	2.18	76.90	1.06	82.80	0.87	82.90	0.98
	78.10	*0.84*	**69.20**	*1.17*	**77.50**	*0.87*	**78.90**	*1.39*	**79.50**	*1.81*
EXTEM Angle										
	75.70	1.61	54.50	2.37	77.60	1.11	80.00	1.12	80.00	1.03
	71.70	2.28	53.50	3.58	77.10	1.93	79.50	1.40	77.40	1.23
	75.90	*1.28*	**55.90**	*1.29*	**79.70**	*0.76*	**80.90**	*0.83*	**79.30**	*1.28*
Platelet Count										
	647.08	69.40	324.69	142.60	440.17	206.31	518.62	187.60	728.43	237.57
	553.37	61.21	182.05	23.88	247.66	42.08	386.39	58.59	549.27	70.68
	562.00	*60.10*	**153.48**	*48.70*	**188.47**	*56.65*	**355.45**	*49.42*	**447.27**	*39.94*
Fibrinogen										
	208.10	11.50	121.30	7.13	234.10	10.80	363.60	38.51	294.20	42.12
	181.80	28.26	110.00	19.96	237.30	19.12	306.90	37.41	271.60	45.59
	176.80	*9.19*	**127.00**	*13.66*	**220.30**	*17.94*	**237.50**	*43.95*	**192.80**	*47.59*
Coll/Epi PFA										

	189.70	22.02	300.00	0.00	276.20	15.91	225.10	21.39	215.11	24.47
	225.80	20.32	296.00	4.00	261.20	20.53	216.80	19.32	183.20	20.44
	227.50	*26.87*	**291.00**	*18.03*	**239.40**	*30.14*	**184.40**	*25.86*	**225.00**	*9.12*
Coll/ADP PFA										
	70.50	4.55	185.40	26.80	150.30	20.24	113.40	21.27	70.89	2.35
	74.60	5.32	132.50	13.07	143.20	16.27	85.90	5.68	96.60	13.52
	74.40	*6.37*	**171.60**	*5.24*	**114.50**	*9.14*	**127.00**	*25.37*	**78.60**	*8.28*
FOT (min)										
	7.00	0.63	8.50	0.73	7.50	1.19	7.80	0.90	7.10	0.81
	7.10	0.99	6.20	0.81	7.70	1.39	5.40	0.98	4.90	0.87
	7.80	*1.02*	**10.70**	*0.97*	**8.40**	*1.11*	**5.90**	*1.24*	**6.00**	*0.98*
CEM (kdynes/cm2)										
	44.36	4.38	4.41	1.43	38.07	5.20	58.77	7.26	58.05	5.43
	37.67	4.71	33.44	6.46	40.58	6.78	76.37	10.66	76.42	8.40
	38.69	*3.38*	**2.55**	*5.46*	**47.72**	*6.30*	**70.38**	*5.99*	**57.01**	*6.45*
PCF (kdynes)										
	10.84	1.57	2.54	0.66	6.71	1.31	12.61	1.58	13.31	1.21
	8.11	1.21	7.04	1.69	9.44	1.95	17.93	2.67	20.16	2.65
	9.64	*1.76*	**1.66**	*1.55*	**8.42**	*1.99*	**13.78**	*1.66*	**12.32**	*2.22*
Liver Enzyme										
	18.80	2.18	13.40	0.88	32.25	4.12	27.22	3.62	19.43	1.81
	19.50	1.11	14.30	1.02	36.30	3.84	35.10	2.79	23.89	1.96
	18.50	*1.46*	**12.89**	*0.73*	**29.22**	*3.53*	**28.20**	*5.66*	**20.80**	*2.67*
Platelets CD62p										
	27.20	3.04	14.39	1.01	19.74	2.04	41.75	4.55	33.15	3.50
	21.11	3.59	15.53	0.73	23.23	2.75	34.23	4.00	34.98	4.86
	30.39	*3.67*	**23.26**	*2.89*	**25.55**	*4.11*	**26.87**	*5.14*	**28.03**	*4.08*
vWF: Ag										
	106.70	4.29	81.90	6.49	81.03	8.32	109.44	13.79	100.70	10.90
	96.90	8.06	86.70	3.65	83.70	2.42	111.10	5.94	111.60	4.45
	75.90	*4.69*	**65.70**	*4.43*	**88.00**	*5.65*	**99.70**	*7.92*	**103.10**	*3.10*
Collagen Agg (Ohms)										
	9.70	0.43	6.05	0.44	8.60	0.43	9.80	0.76	10.95	0.63
	8.85	0.85	7.40	1.56	8.25	0.72	9.05	0.70	10.20	0.88
	9.39	*0.56*	**3.78**	*0.69*	**6.06**	*1.04*	**8.50**	*0.84*	**10.50**	*0.86*

ADP Agg (Ohms)	8.70	0.47	6.95	0.51	8.25	0.41	9.80	0.93	10.20	1.16
	8.05	0.87	7.35	1.00	7.55	0.81	9.95	1.34	8.85	0.88
	8.83	*1.33*	**6.85**	*0.64*	**9.00**	*1.26*	**10.35**	*0.36*	**11.64**	*0.89*
CAT lag										
	5.63	0.56	7.43	0.58	6.03	0.49	7.70	0.72	7.14	1.01
	6.28	0.74	7.46	0.69	6.01	0.23	6.86	0.77	7.38	0.76
	4.99	*0.19*	**8.51**	*0.40*	**6.83**	*0.85*	**7.95**	*0.40*	**7.45**	*0.98*
CAT ETP										
	1218.76	116.27	815.67	103.42	841.82	117.71	1366.51	134.14	1472.42	111.00
	924.08	130.18	1062.92	133.12	1073.74	140.66	1151.05	205.07	1537.37	103.81
	1141.66	*106.75*	**732.11**	*100.08*	**1032.55**	*95.53*	**1404.96**	*44.68*	**1357.70**	*139.30*
CAT Peak										
	71.94	9.10	43.72	5.92	39.06	6.26	87.81	10.18	99.21	12.41
	52.15	10.66	59.56	8.63	55.62	8.60	82.49	11.20	103.10	8.85
	74.67	*9.31*	**38.16**	*9.75*	**52.25**	*6.63*	**88.98**	*7.88*	**97.28**	*10.97*
CAT Tip										
	16.01	0.86	20.53	0.48	18.75	1.15	17.72	0.66	16.50	0.92
	17.59	1.73	18.48	1.12	17.72	1.07	29.99	12.52	16.99	1.13
	15.62	*0.92*	**20.93**	*0.92*	**19.80**	*0.81*	**17.85**	*1.14*	**16.54**	*1.15*
Neutrophils										
	234.00	48.00	189.20	25.35	173.86	14.79	185.86	20.10	211.43	28.86
	202.22	44.85	272.31	68.08	240.22	33.20	306.00	71.80	181.39	27.94
	107.406	*15.03*	**466.25**	*15.37*	**393.5714**	*16.90*	**521.8571**	*27.16*	**575.7143**	*22.59*
Monocytes										
	247.50	39.74	176.50	3.50	186.50	21.40	230.17	26.70	217.20	32.98
	199.20	32.08	218.67	28.07	226.57	30.14	272.43	39.19	235.38	32.42
	93.17	*4.31*	**211.40**	*3.90*	**259.13**	*3.91*	**327.86**	*3.38*	**274.67**	*3.33*
Natem TPI										
	92.54	12.24	30.59	3.68	61.12	8.21	91.43	16.68	113.81	14.43
	69.77	9.98	41.30	10.97	80.63	14.69	138.94	22.63	175.08	26.56
	73.43	*11.93*	**35.61**	*6.43*	**63.94**	*13.46*	**119.00**	*9.29*	**109.68**	*17.36*
Intem TPI										
	194.49	24.54	44.85	7.57	157.90	20.84	326.06	37.91	384.92	64.29

	173.74	37.08	57.35	19.21	166.84	28.99	307.89	30.61	326.92	24.86
	220.43	*23.22*	**51.97**	*24.89*	**209.47**	*28.87*	**279.61**	*37.22*	**290.87**	*55.00*
Extem TPI										
	140.35	20.74	30.86	4.07	121.23	14.53	378.93	85.34	327.02	84.12
	125.13	36.34	37.13	11.80	140.59	28.52	301.59	47.22	237.75	47.11
	138.16	*15.35*	**36.76**	*11.29*	**196.58**	*23.92*	**260.94**	*26.03*	**209.13**	*62.15*

	Perftoran
	Saline
	Control

Biomarkers results

Part 1: Biomarkers of Neuronal Damage in Hemorrhage Sheep:

Alpha-II spectrin is a 280 kDa cytoskeletal protein found in neurons whose breakdown products are established biomarkers of neuronal damage. Connected with necrosis or apoptosis, calpain or caspase-3 cleave alpha-II spectrin into different sized segments. Necrotic induced calpain activity is associated with 150 and 145 kDa breakdown products. Apoptosis induced caspase-3 activity is associated with 150 and 120 kDa breakdown products. Alpha-II spectrin breakdown products have been detected in brain tissue, cerebrospinal fluid, and plasma, and thus can serve as a minimally invasive method of analyzing potential brain damage and efficacy of PFC treatment.
Male sheep (20-30kg) were anesthetized, intubated, and ventilated on room air with 1-2% isoflurane. Animals were instrumented for measurement of vitals, hemodynamics, and sampling of blood for gases, biochemistry, and hematologic evaluation. Arterial blood was removed 3ml/kg/min until MAP below 40mmHg. Once MAP returned to 50 (or 15min), blood was removed 2ml/kg/min until MAP below 35mmHg. Once MAP returned to 40 (or 15min), blood was removed 1ml/kg/min until MAP below 25mmHg. Sheep remained at MAP 25-30mmHg for 1hr. Sheep were then resuscitated with minimal volume hetastarch (until MAP 60mmHg) then given either 6cc/kg saline (n=8) or Oxygent PFC (n=8). Control animals (n=8) underwent the same procedures as hemorrhagic sheep except blood was not withdrawn nor fluid resuscitation administered. Plasma samples were collected 24 hours prior to hemorrhage (baseline) and at 24 hours, 4 days, and 7 days post hemorrhage. Plasma samples proteins were balanced by UV-Visible spectrophotometry, resolved by SDS-PAGE, and transferred to nitrocellulose. The resulting western blots were probed for Alpha-II spectrin breakdown products using a commercially available antibody, visualized by chemiluminescence, and densitometerically analyzed. Figures 1-4 show western blots of plasma samples from control, hemorrhage sheep treated with PFC, and hemorrhage sheep treated with saline at baseline, 24 hours, 4 days, and 7 days following hemorrhage. Analysis of the blots between 100 and 150 kDa at all time points in all samples does not show any expression of Alpha-II spectrin breakdown products.

Figure 1

Baseline

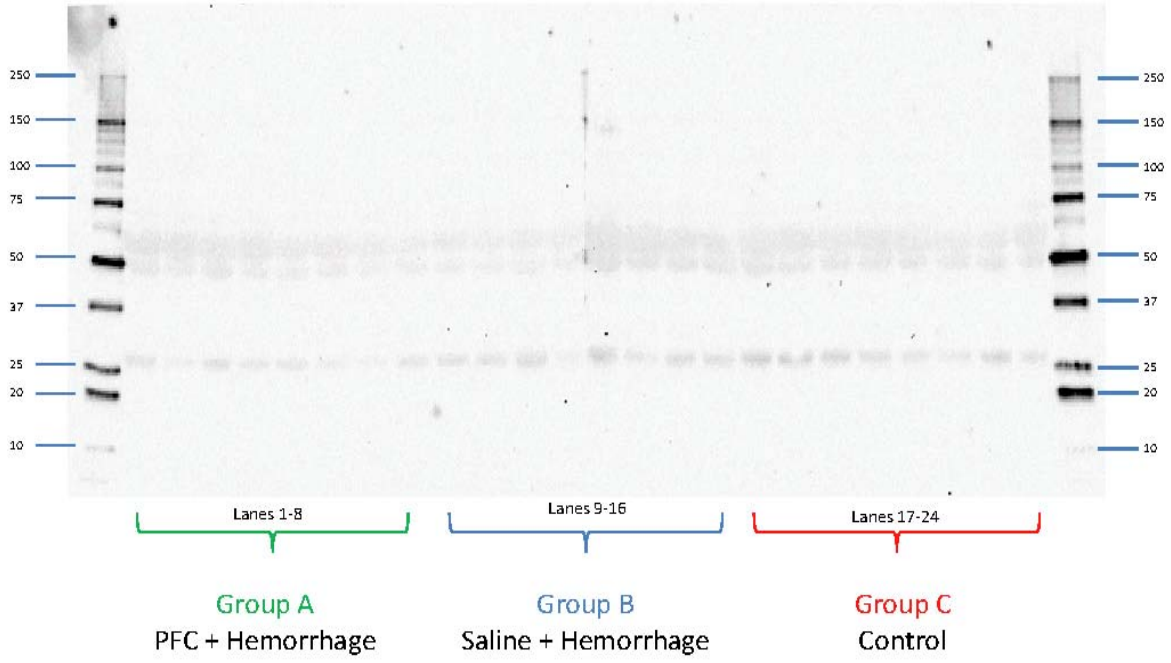


Figure 2

24 Hours

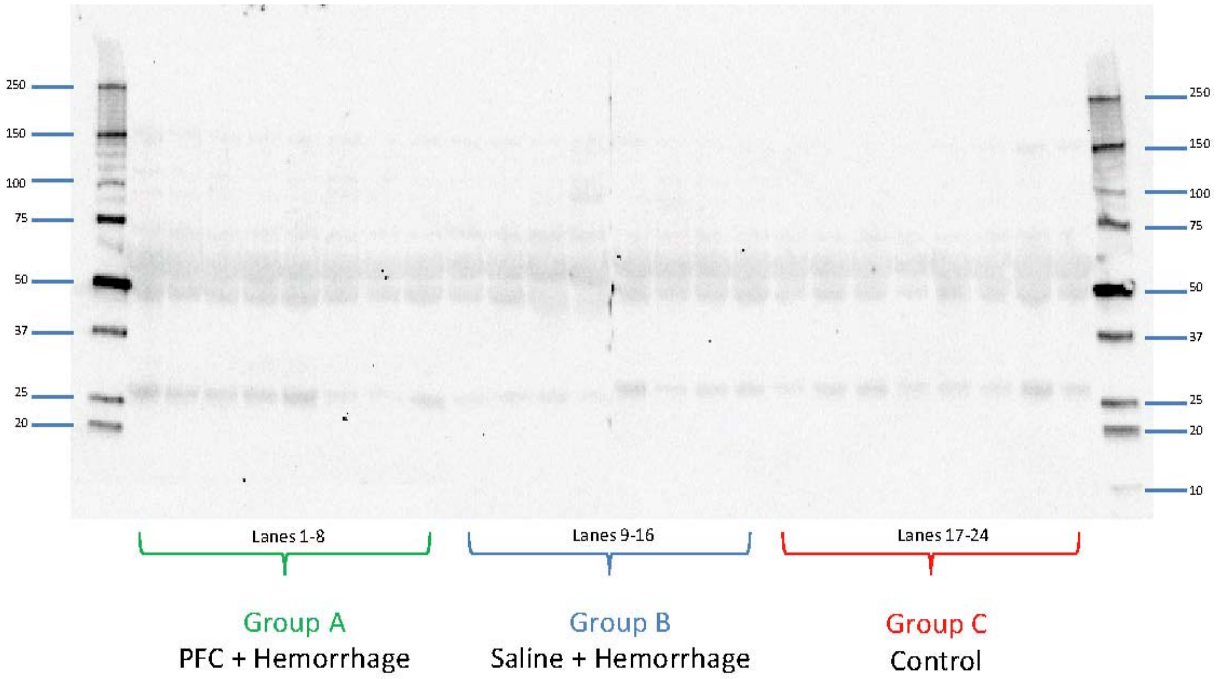
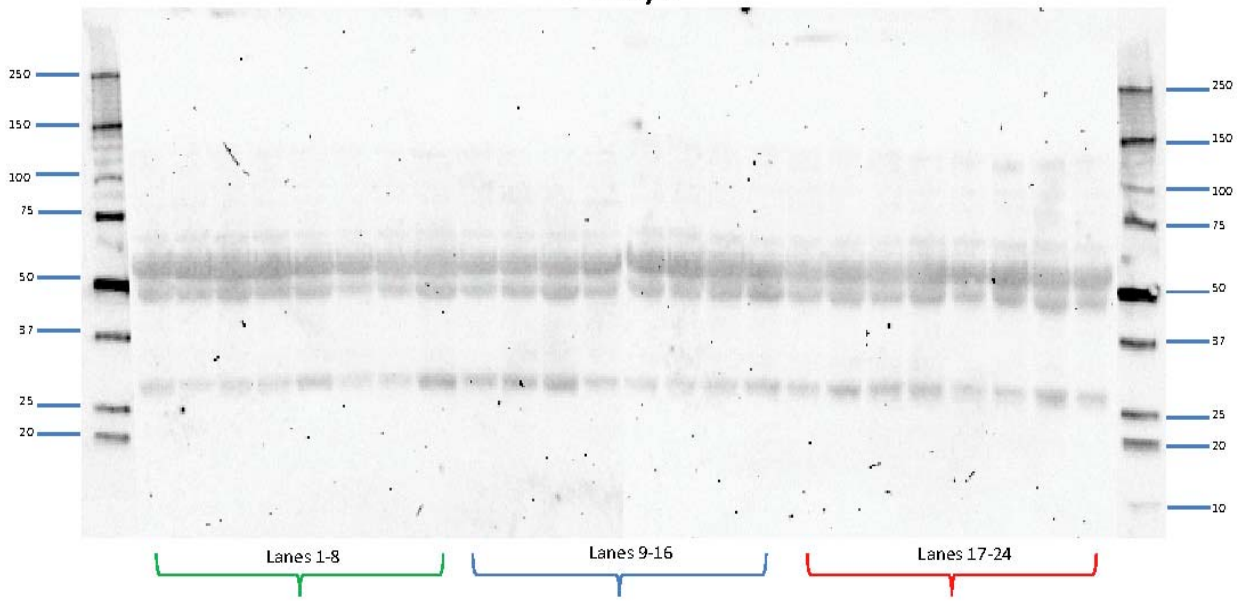


Figure 3

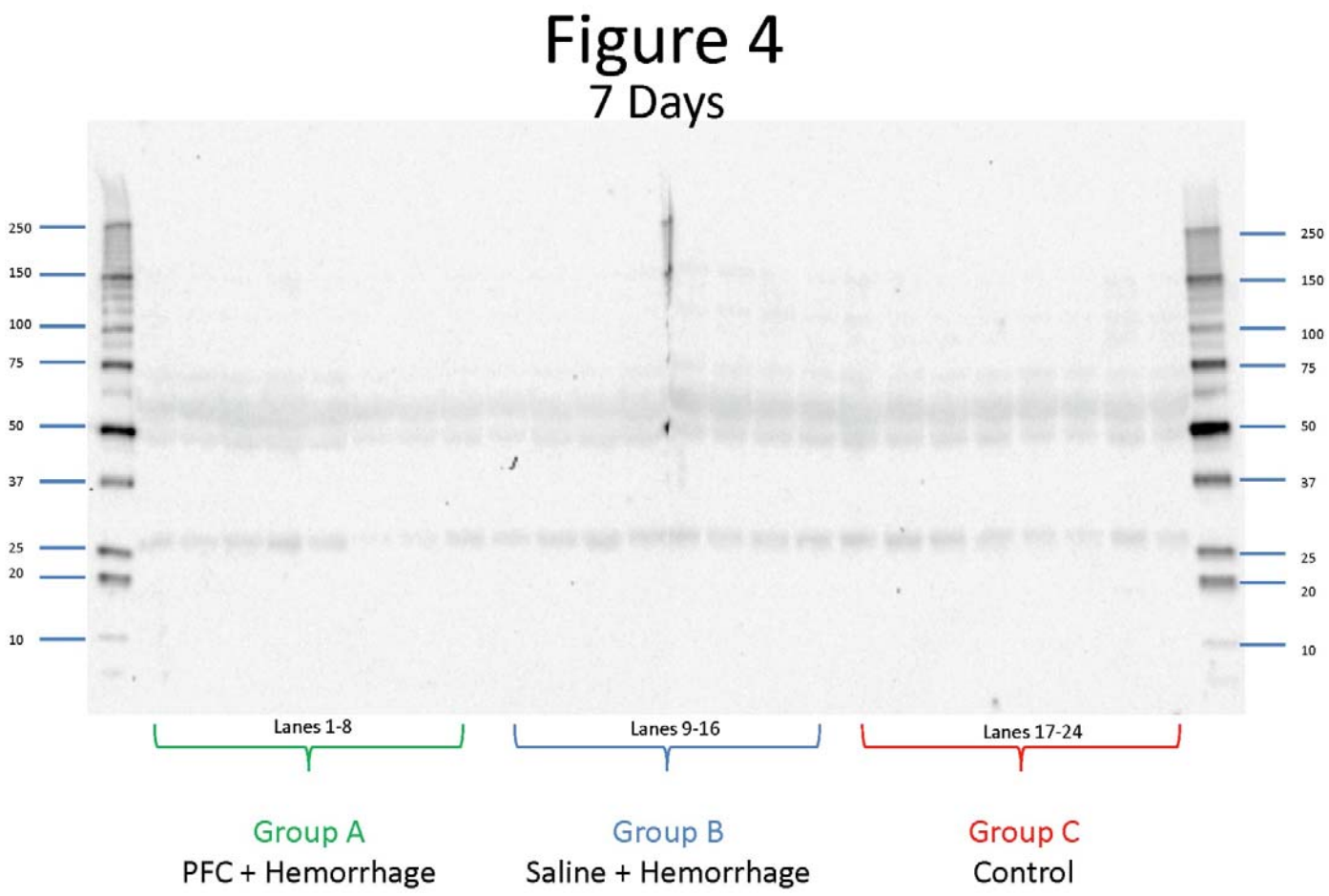
4 Days



Group A
PFC + Hemorrhage

Group B
Saline + Hemorrhage

Group C
Control

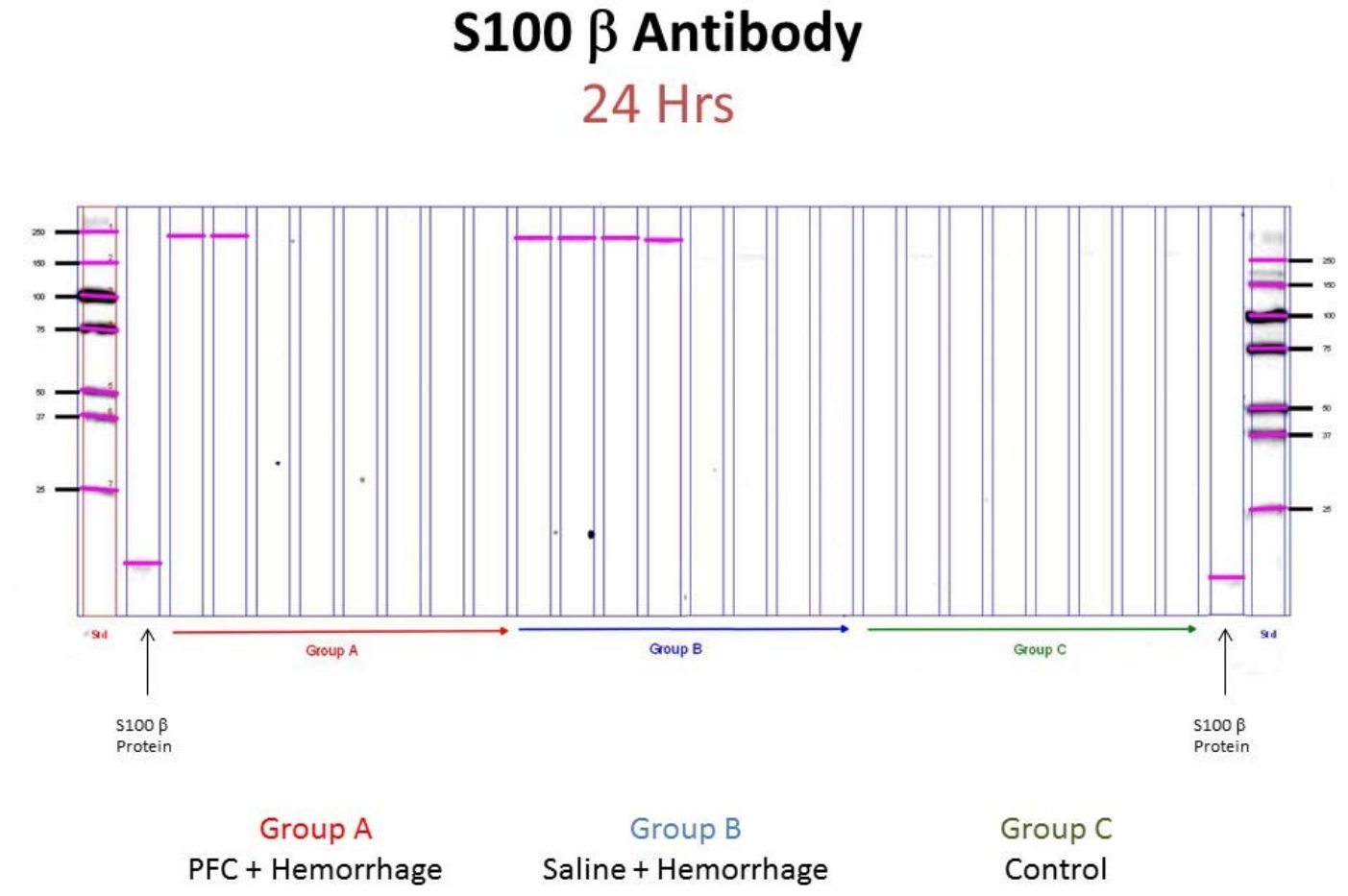


Part 2: Biomarkers of Neuronal Damage in Hemorrhage Sheep:

S100β is a 21 kDa Ca^{2+} binding homodimer of the S100 family of multifunctional proteins with regulatory roles in a variety of cellular processes. S100β is the most studied of the S100 family and it is often utilized as a marker of global brain injury (due to its abundance in astrocytes and cellular release from astrocytic activation) and an indicator of blood-brain barrier integrity (often found in csf and blood following multiple brain pathologies). S100β can be detected in serum/plasma and CSF samples and is negatively correlated with outcome associated. S100β has been detected in brain tissue, cerebrospinal fluid, and plasma, and thus can serve as a minimally invasive method of analyzing potential brain damage and efficacy of PFC treatment.

Male sheep (20-30kg) were anesthetized, intubated, and ventilated on room air with 1-2% isoflurane. Animals were instrumented for measurement of vitals, hemodynamics, and sampling of blood for gases, biochemistry, and hematologic evaluation. Arterial blood was removed 3ml/kg/min until MAP below 40mmHg. Once MAP returned to 50 (or 15min), blood was removed 2ml/kg/min until MAP below 35mmHg. Once MAP returned to 40 (or 15min), blood was removed 1ml/kg/min until MAP below 25mmHg. Sheep remained at MAP 25-30mmHg for 1hr. Sheep were then resuscitated with minimal volume

Figure 1



hetastarch (until MAP 60mmHg) then given either 6cc/kg saline (n=8) or Oxygent PFC (n=8). Control animals (n=8) underwent the same procedures as hemorrhagic sheep except blood was not withdrawn nor fluid resuscitation administered. Plasma samples were collected 24 hours prior to hemorrhage (baseline) and at 24 hours, 4 days, and 7 days post hemorrhage. Plasma sample proteins were balanced by UV-Visible spectrophotometry, resolved by SDS-PAGE, and transferred to nitrocellulose. The resulting western blots were probed for S100β

using a commercially available antibody, visualized by chemiluminescence, and densitometerically analyzed. Figure 1 shows western blot of plasma samples from control, hemorrhage sheep treated with PFC, and hemorrhage sheep treated with saline at 24 hours following hemorrhage. Analysis of the blot at 10.5 kDa at all time points in all samples does not show any expression of S100β. The results suggest that there is no astrocytic activation nor breakdown of the blood brain barrier 24 hours following hemorrhagic shock in the currently utilized sheep model. This data further supports the lack of detection of alpha II spectrin breakdown products at all time points in this model previously reported in the 2015-July-15 quarterly report.

Sheep Hemorrhagic shock procedures

Hemorrhage Model Development Protocol

Sheep Arrival Day: (24 hours after arrival)

1. Check the sheep in general: standing, walking, eating, defecation (stool in normal)/urination, any cough, running nose, sneezing. Also, check whole body for any ulcer or damage.
2. Check the sheep vital sign: heart rate, temperature, respiratory rate (HR, T, RR)
3. Draw blood for CBC (HM5) and get a stool sample for DAR.
4. Document and inform DAR the initial examination.

Prep Work (Day Before)

1. Separate two sheep (one will be used and the other won't) from the flock to have them fast (only water) for 24-48 hours.
2. Be sure to place Fasting Signs to the cages and note it on their USDA sheets
3. Check gas tanks, both on the cart and in B3-030 (O2, N2, Room Air and E-tanks). As well as the Isoflurane levels in both the ventilator and on the cart.
4. Prepare surgical packs for both surgeon 1 and 2. (see index for pack content)
5. Sterilize (or make sure there are) 4 packs of gauze, and 2 beakers (see index for instructions)
6. Check expiration dates on equipment, sterile materials, drugs and chemicals
   - -Maxi-Cide
   - -Maxi-zyme
   - -Isoflurane
   - -KCl
   - -Lidocane
   - -Solutions in ABL and HM5
   - -Disks for the VetScan
7. Soak in Maxi-Cide: 3- PE90, 1 -PE160, and 1-PE190, 1- SwanGanz, 10-12 stopcocks and a set of small spring scissors, a long line for hemorrhage and two long lines for infusion
8. Stock the room with Syringes (60mL, 10mL, 5mL and 1mL), Needles (18G, 20G), Gauze, Catheters, etc..
9. Take the ABL machine out of standby (allow it to calibrate)

The Morning of Surgical Experiment, Room B3-032

1. Turn on the computers, one to run the BioPac and the other to run excel
2. Calibrate the gasses, pressure sensors and Pneumotac.
3. Using sterile technique drain the Maxi-Cide (from the soaked catheters) and fill it with saline
4. Ready sterile gowns, 10-10mL syringes (sterile), 20-1mL syringes (sterile), 1-bottle of saline (sterile) , 2 blades (size 22, 10), and Suture material (3-0 Monocryl)
5. Prepare Maxi-Zyme solution for cleaning tools
6. Sync all clocks, Computer, ABL, HM5, VetScan and wall clock
7. Double check the ABL, HM5 and VetScan that they are ready to run samples. Run a blank on the HM5
8. Remove 4 vials of Pfc if it will be used for resuscitation that day (5mL/Kg)

Retrieve the Sheep (using the cart), Animal Room on B2

1. Check the Vital Signs of all the sheep
2. Give the sheep a dose of Carpofen (Dose 3mg/Kg)
3. Record Time for Start of Isoflurane and Time of consciousness loss
4. Use the nose cone on the cart (O2 up to 8 L/min, Isoflurane up to 4-5%) to knock the sheep unconscious, put the sheep on the cart and lower the Isoflurane levels to 2-3%.
5. Watch sheep closely to make sure he is continuously breathing
6. Make sure to weigh animal (kg) before leaving B2.

7. Move sheep to B3-032 (using back freight elevator)
8. Start the BioPac
9. Put a trachea tube in the Sheep and move to the ventilator (Ventilator output shows: 21% O2, 79% N2) put the Iso up to 4% initially and move down to 1.5% once the breathing becomes regulated. (regardless of O2 Saturation levels) Flow rate is 6-8mL/min/Kg
10. Place the Gastric tube and secure the trachea tube with umbilical tape, Place pillow under neck and a drip tray to catch saliva. (see picture in index)

Prepping the Sheep

1. With the sheep on his back (supine) strap his limbs down
2. Shave his neck, limbs (for electrodes), and groin
3. Place ECG electrodes on 3 limbs: Front Left=Black, Front Right= White, and Back Left= Red
4. Clean shaved skin with 70% alcohol spray and gauze, and temperature probe, Turn on water blanket to 40^{o}, add a second blanket if the sheep's temperature drops to below 38^{o}.
5. Clean the groin area with betadine, and use a Sharpie to mark incisions

Prepping for Surgery

Surgeons

1. Before scrubbing in make sure to have shoe covers, hair cover and magnifying glasses on
2. Scrub in by sterilizing hands with a surgical scrub kit.
3. With assistance put on a sterile gown and gloves
4. Place sterile drapes on the sheep and cut access slits, arrange tools, syringes, beakers and catheters on the tray

Non sterile staff

1. Assist in the scrub in procedures.
2. Adjust light source
3. Place the tool trays and put the sterile packs on top, open the non-sterile outer wrapping with out touching the inner wrappings.
4. With Assistance from a sterile person set the tools, place and fill two beakers with sterile saline
5. Drop syringes (10-10mL, 20-1mL, 5-5mL), 2 blades (22), catheter and suture material onto the sterile field

Sterile Surgical Procedures

Surgeon #1 (Left Leg)

1. Cut down and catheterize the Femoral Artery with a PE-90 or PE-160 catheter (this will be for the hemorrhage)
2. Separate the muscle tissue to get to the Femoral Vein to place the Swan-Ganz (7.5)
3. With Assistance from a non-sterile tech connect the Swan-Ganz to the CVP, PAP and the Cardiac Output Machine.
4. Calibrate the Cardiac Output, take a MV blood sample to get the ctHb value convert from g/dL to mmol/L.
5. Once nemotac is running take an arterial sample and a mixed venous sample and run the ABL machine.

Surgeon #2 (Right Leg)

1. Cut down and catheterize both a branch of the Femoral Artery and a branch of the Femoral Vein (not touching the nerve) each with a PE90 catheter.
2. Connect the Arterial line to the AP sensor
3. With assistance from on Sterile Tech, pull 1mL of Heparin and heparin 20 1 mL syringes
4. Collect a blood sample to run a baseline CBC and ABL
5. Hang a bag of saline to help with water loss (saliva)

Non Sterile Tech

1. Assist with surgeries when needed
2. Document all blood draws, Catheter placements and any vitals in the BioPac
3. Every 15 minutes a toe pinch or eye reflex needs to be done and documented on the excel spread sheet as well as the Heart Rate and O2 saturation levels

After Surgical Procedures have been completed you begin stabilization, you can take the Baseline samples at this time.

Pre Hemorrhage (1 Leg)

1. Ready the pump, One set of line ready with adapters on both ends, a sterile stop-cocks and a sterile stop-cocks, one set of sterile lines for drawing of blood
2. Set the speed and rate based on the first step
3. A sterile tech needs to connect a stop-cock and the three lines to the open arterial catheter (left) while the non sterile tech connects the lines to the pump

Hemorrhage

Hemorrhage Techs, One is Sterile and One is Non-Sterile

1. Mark the time at the beginning of hemorrhage
2. Starting with the first rate step(3mL/min/Kg), pull blood while monitoring the mean arterial pressure (MAP). Stop hemorrhaging when either the MAP drops to 40mmHg or the Hemorrhage goal has been reached.
3. If goal has not been reached but the MAP drops to below 40mmHg you wait until the MAP rises back up to 50mmHg or it has been stable for 15 minutes.
4. Move to the second rate step (2mL/min/Kg) and continue to pull until either the MAP drops to 35mmHg or the Hemorrhage goal has been reached.
5. If goal has not been reached but the MAP drops to below 35mmHg you wait until the MAP rises back up to 40mmHg or it has been stable for 15 minutes.
6. Move to the third rate step (1mL/min/Kg) and continue to pull until either the MAP drops to a minimum or the Hemorrhage goal has been reached.
7. If goal has not been reached but the MAP drops to below 25mmHg you wait until the MAP rises back up to 30mmHg or it has been stable for 15 minutes.
8. Continue pausing and restarting at the Third rate until either the goal has been reached or the time of hemorrhage has reached an hour.

Non-Hemorrhage Tech

1. Keep track of times for when the hemorrhage starts and pauses
2. document in the Biopac when hemorrhage starts, stops and pauses as well as when rates are changed
3. make sure the hemorrhage team keeps to script, and that blood samples are taken
4. document when blood samples are taken and when catheter lines are flushed.

Shock

1. Hold stable for an hour while monitoring the MAP
2. Take blood samples right after hemorrhage and right before Resuscitation
3. Continue hemorrhage if MAP goes above a preset limit (30-25 mmHg)
4. At desression of the PI- ultimate goal is MAP
5. Sterile Tech can prepare 1mL syringes with heparin for blood samples and get lines prepped for resuscitation
6. Non sterile tech should ready the syringe pump (rate is 60mL/min for 15 min)

Resuscitation

1. Push sterile syringes of chosen drug (saline, hetastarch or pfc) in two catheters at once (Right Vein and Left Swan Gantz) Until the MAP becomes stable at a preset height(60mmHg).

2. Watch vitals for an hour taking blood samples at 0 post resuscitation and every 20mins after until you hit the hour mark

Wake up the Sheep

1. (sterile) Remove all the catheters, and tools. Suture up skin and use betadine on the sutures.
2. remove restraints and temperature probe, as well as ECG sensors
3. Turn off isoflurane and begin weaning sheep off of the ventilator until he gains auto breath
4. Remove Trachea tube and gastric tube
5. put on the cart and bring back up to animal pens (under anestheisa on the cart)
6. monitor sheep closely as it wakes up in the pen(isolated)

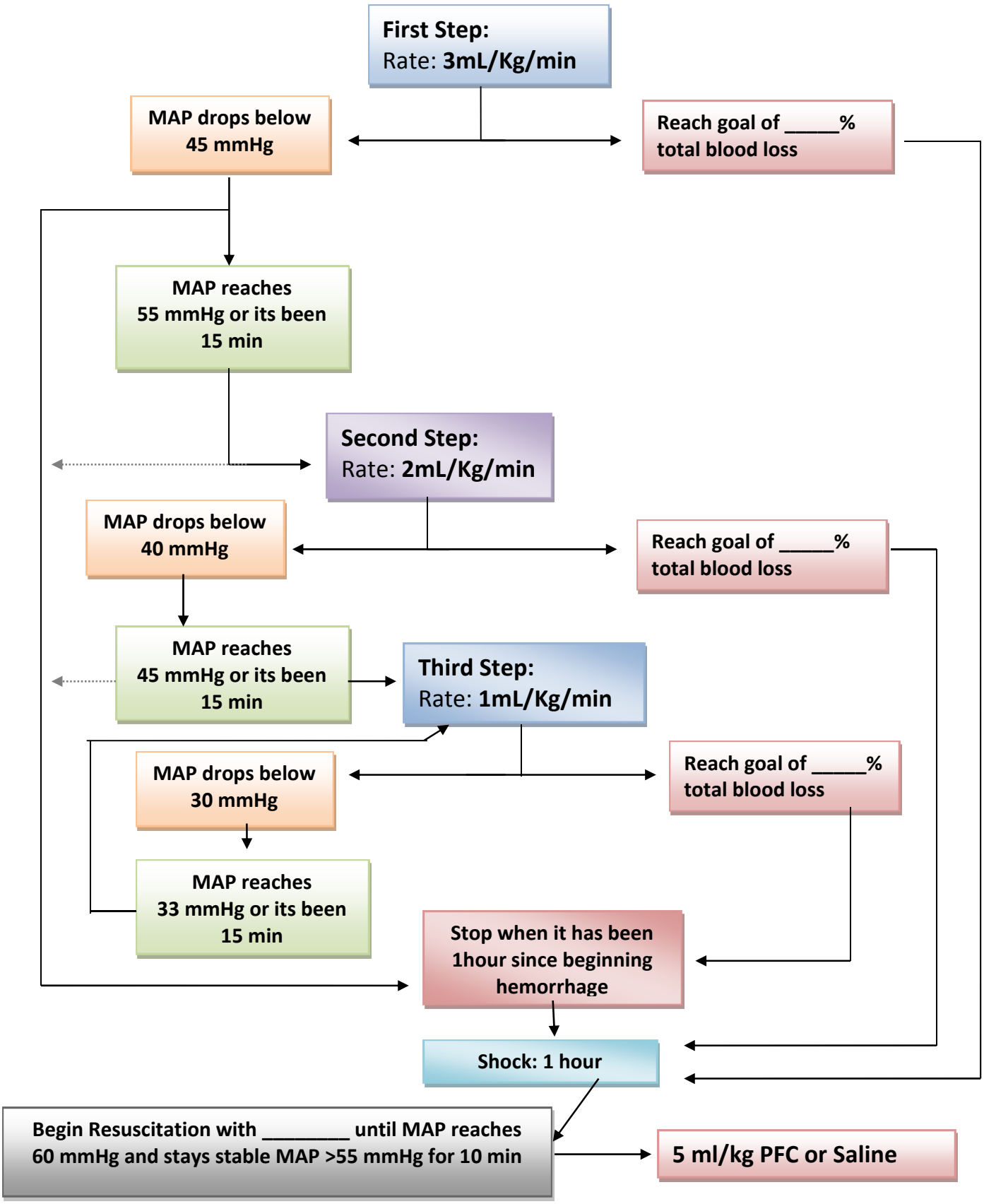
Clean up

1. Clean all surgical tools, catheters and let them air dry
2. remove soiled drapes and chucks from the room (place in red bin)
3. Assure all machines are turned on standby and that all gas tanks are off
4. Clean all surfaces with _____ (as per Q-Fever policy)
5. Make sure to dispose of gastric juices and clean all tubing (ventilator and G-tube)
6. Sweep/mop floors and remove any trash (red bin included)
7. Make sure all sharps are disposed of properly

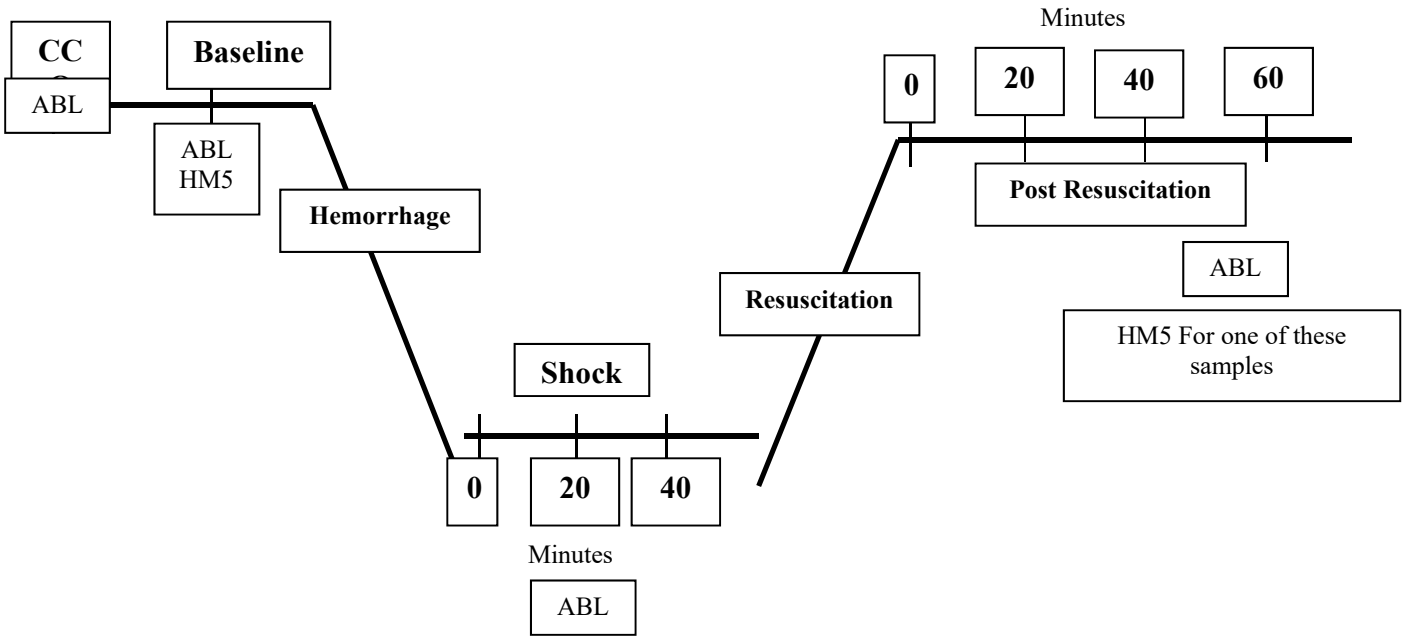
Daily Sheep Health Observations

1. Every morning before 10am each sheep (wither control or not) will have their vital signs checked.
2. Check, Heart Rate, Temperature and Rate and record daily
3. Take note of social behavior, eating and drinking habits, as well as urination and excretion
4. Look at Surgical areas and give a dose of Carpofen every day (24hrs) for 3 days.

Index: Hemorrhage Flow Chart

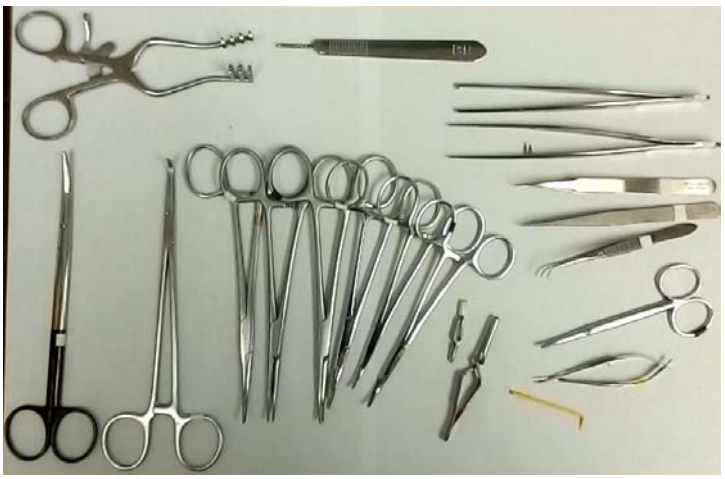


Index: Blood Sample Chart



Index: Sterile Technique

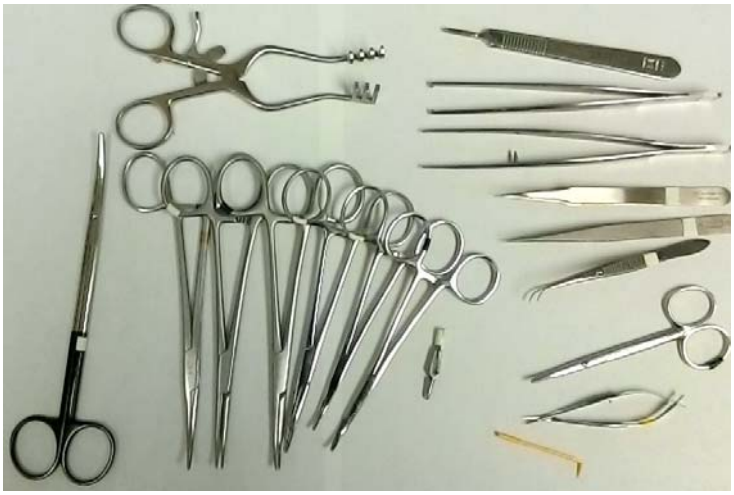
Sterile Kits: (each kit has to have an indicator strip on the inside and one on the outside, and Labeled)
How to fold:
Surgeon #1



- 1 Retractor (Weitlaner-Locktite)
- 1 Scalpel Handle
- 1 large curved Scissors (Metzenbaum)
- 1 90º Hemostat
- 6 Curved Hemostats
- 1 Small bulldog clamp
- 1 Larger bulldog clamp
- 1 Inducer
- 1 set of small spring scissors
- 1 small curved scissors
- 2 sharp forceps
- 1 curved forcep (small)
- 1 tissue forcep with teeth
- 1 tissue forcep with ridges

In Surgical Pack
- Drape for surgery
- extra drape to place on top of the tray
- gauze 4x4 and 2x2
- (6-8 pieces) 0-3 silk
- Autoclave strip

Surgeon #2



- 1 Retractor (Weitlaner-Locktite)
- 1 Scalpel Handle
- 1 large curved Scissors (Metzenbaum)
- 6 Curved Hemostats
- 1 Small bulldog clamp
- 1 Inducer
- 1 set of small spring scissors
- 1 small curved scissors
- 2 sharp forceps
- 1 curved forcep (small)
- 1 tissue forcep with teeth
- 1 tissue forcep with ridges

In Surgical Pack
- Drape for surgery
- extra drape to place on top of the tray
- gauze 4x4 and 2x2
- (6-8 pieces) 0-3 silk
- Autoclave strip

Gauze

Beakers

Drapes

Catheters

Prepared Surgical Table

Abstracts of MHSRS and National Conference

Title: EVALUATION OF NONINVASIVE CARDIAC OUTPUT MONITORING IN SHEEP WITH HEMODYNAMIC INSTABILITY

Authors: Paul A. Middleton, MD, Penny S. Reynolds, PhD, Jiepei Zhu, PhD, Bruce D. Spiess, MD

INTRODUCTION: Thermodilution-based cardiac output (T-CO) monitoring requires insertion of a pulmonary artery catheter (PAC), and is therefore highly invasive with inherent risk of complications. In contrast, non-invasive cardiac output monitoring (NICOM) with thoracic bioreactance is discreet, and sensors are easy to place. This technique measures the phase shift between an applied alternating current and voltage measured across the thorax; phase shifts are tightly coupled to changes in thoracic pulsatile blood flow, and hence stroke volume.[1] This technology has been validated in several non-trauma and trauma patient populations, but not in the setting of acute hemorrhagic shock.[2] In this study, we evaluated the precision and accuracy of NICOM against thermodilution cardiac output (continuous PA catheter: T-CO) under hemodynamically unstable conditions. The objective was to compare the effect of intravascular volume depletion on measurements obtained by NICOM versus the gold standard PAC. The hypothesis was that bioreactance measured CO was able to follow very low CO when PA catheter monitoring might be unable to obtain data.

METHODS: The experimental protocol was reviewed and approved by both the Animal Care and Use Committee (IACUC) of Virginia Commonwealth University, Richmond, VA and the Animal Care and Use Review Office (ACURO), Office of Research Protection, US Army Medical Research and Materiel Command (MRMC), Fort Detrick, MD. All experiments included animal handling and procedures were supervised by the veterinarians of Division of Animal Resources (DAR) in VCU, which were following The Guide for the Care and Use of Laboratory Animals (8th edition, National Research Council (US) Committee, National Academies Press (US), 2011). Twenty male juvenile Dorset or Dorper sheep (mean 21 kg, SD 2.2 kg) being studied for a hemorrhagic shock model of resuscitation utilizing perfluorocarbon (PFC)/placebo in a protocol had the bioreactance technology added to the already planned PA catheter monitoring (Edwards Lifesciences VGSV Vigilance Monitor, Irvine, CA, USA). Animals were anesthetized with 5% isoflurane and maintained with 1~2% isoflurane during the procedure. Animals were intubated (30F tracheal tube) and ventilated using Dräger Fabius GS Anesthesia Machine (Dräger Medical Inc., Telford, PA, USA) with 30% O2:70% N2. The animals' femoral arteries (16G) and veins (18G) were placed with catheters including a 7.5F Swan-Ganz CCOmbo V for hemodynamic monitoring and resuscitation. Hemodynamic monitoring included arterial blood pressure (BP), and mean arterial blood pressure (MAP), pulmonary artery pressure (PAP), central venous pressure (CVP) and continuous CO. The NICOM system (Cheetah Medical, Wilmington, DE, USA) requires placement of four double electrode stickers on the thorax. Locations were shaved, then upper stickers were placed across the mid-left and right clavicle, and lower stickers were placed just below the mid-left and right last rib. Animals were stabilized for 15 min then hemorrhaged (controlled arterial bleed) following a stepwise hemorrhagic shock model for sheep: bleeding 35~50% total blood volume, MAP <33 mmHg for 60 minutes (class III shock model). The first fast bleeding phase: 3ml/kg/min until MAP <45 mmHg; the second bleeding phase: 2 ml/kg/min until MAP <40 mmHg starting at MAP recovered to 55 mmHg or 15 minutes after the first fast bleeding. The third bleeding phase: 1 ml/kg/min until MAP <30 mmHg (±3mmHg) starting at MAP recovered to 45 mmHg or 15 minutes after the second bleeding. Resuscitation was first intravenously infused hetastarch

(6% hydroxyethyl starch 130/0.4 in 0.9% sodium chloride), equal volume to blood loss. Animals were stabilized for 10 minutes and maintained MAP >55 mmHg before infusing PFC or normal saline (5 ml/kg) over 15 minutes. Animals were observed for 60 minutes after resuscitation completed, then allowed to recover from anesthesia and carried back to DAR facility. The data collected included CO and mean arterial pressure (MAP) every 20 minutes from the acutely hemorrhaged sheep from baseline, to shock, then 1 hour post-resuscitation. Data were compared between measures and over time.

RESULTS: MAP at baseline averaged 68 mmHg; average baseline CO was 2.0 and 2.9 L/min for PAC and NICOM respectively. NICOM CO measurements were consistently higher than PAC (0.85 L/min; SE 0.16 L/min; $p<0.0001$; limits of agreement -1.6, 3.3 L/min). The greatest differences between methods occurred at the end of the shock period, coinciding with the greatest variability in hemodynamic stability (MAP standard deviation 23 mmHg). The overall correlation between PAC and NICOM measurements over the entire shock-resuscitation cycle was moderate ($r = 0.65$); although 14/20 animals showed good to excellent congruence between methods (cross correlation $r > 0.89$ at zero lag), PAC readings for 6/20 sheep consistently lagged behind NICOM. Although NICOM readings had higher intrinsic variability (NICOM = 0.98; versus PAC = 0.13), PAC readings were less reliable with 24 dropped or out-of-range observations (CO << 0.1 L/min) during the shock phase, versus none with NICOM.

CONCLUSION: NICOM outperformed PAC when measuring CO in hemodynamically unstable subjects with rapidly changing MAP. NICOM responses were consistently reliable with acceptable accuracy in a clinically realistic model of a hemodynamically challenging situation. Availability of such a tool will allow clinicians to have information about CO in patient when the T-CO method is not feasible to help diagnose and guide therapy.

References:

1. Raval NY, Squara P, Cleman M, Yalamanchili K, Winklmaier M, Burkhoff D. *Multicenter Evaluation of Noninvasive Cardiac Output Measurement by Bioreactance Technique*. Journal of Clinical Monitoring and Computing 2008; 22: 113-119.
2. Dunham MC, Chirichella TJ, Gruber BS, et al. *Emergency department noninvasive (NICOM) cardiac outputs are associated with trauma activation, patient injury severity and host conditions and mortality.* The Journal of Trauma and Acute Care Surgery 2012; 73: 479-485.

Figure I

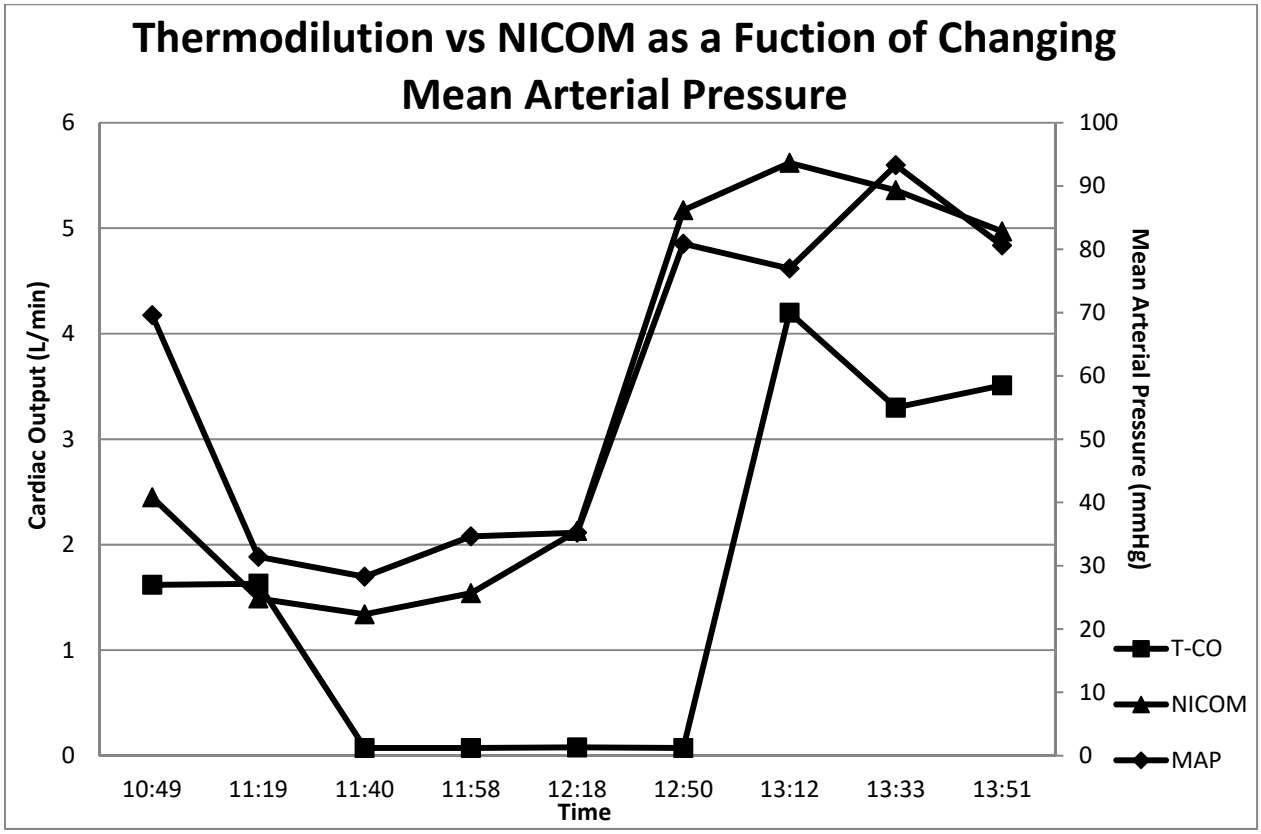


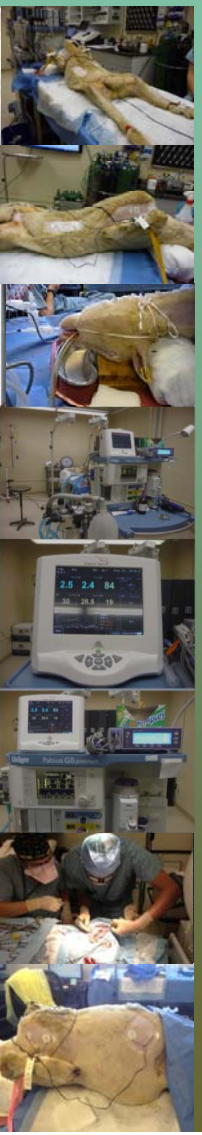
Figure I: MAP and corresponding CO over a period of hemorrhage and resuscitation, demonstrating the unreliable nature of thermodilution at low intravascular volume.

Evaluation of Noninvasive Cardiac Output Monitoring in Sheep with Hemodynamic Instability

Paul Middleton[1], M.D., Jiepei Zhu[1], Ph.D., Penny Reynolds[1], Ph.D., and Bruce D. Spiess[1,2], M.D.

[1]Departments of Anesthesiology and [2]VCU-Jonson Center for Critical Care and Pulmonary Research
School of Medicine, Virginia Commonwealth University, Richmond, VA 23298-0695

IARS Control ID #787



Introduction

Thermodilution-based cardiac output (T-CO) monitoring requires insertion of a pulmonary artery catheter (PAC), and is therefore highly invasive with inherent risk of complications. In contrast, non-invasive cardiac output monitoring (NICOM) with thoracic bioreactance is discreet, and sensors are easy to place. This technique measures the phase shift between an applied alternating current and voltage measured across the thorax; phase shifts are tightly coupled to changes in thoracic pulsatile blood flow, and hence stroke volume.[1] This technology has been validated in several non-trauma and trauma patient populations, but not in the setting of acute hemorrhagic shock.[2] In this study, we evaluated the precision and accuracy of NICOM against thermodilution cardiac output (continuous PA catheter: T-CO) under hemodynamically unstable conditions. The objective was to compare the effect of intravascular volume depletion on measurements obtained by NICOM versus the gold standard PAC. The hypothesis was that bioreactance measured CO was able to follow very low CO when PA catheter monitoring might be unable to obtain data.

Materials & Methods

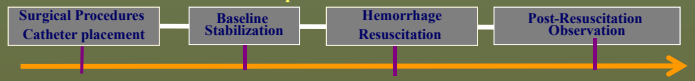
Subjects and Groups:

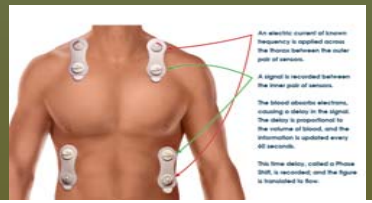
- The experimental protocol was reviewed and approved by the Animal Care and Use Committee of Virginia Commonwealth University
- Twenty male juvenile Dorset or Dorper sheep (mean 21 kg, SD 2.2 kg) being studied for a hemorrhagic shock model of resuscitation utilizing perfluorocarbon (PFC)/placebo in a protocol had the bioreactance technology added to the already planned PA catheter monitoring (Edwards Lifesciences VGSV Vigilance Monitor, Irvine, CA, USA).

Hemorrhagic Shock and Resuscitation Procedures:

- Animals were anesthetized with 5% isoflurane and maintained with 1~2% isoflurane during the procedure.
- Animals were intubated (7.5F tracheal tube) and ventilated (Dräger Fabius GS Anesthesia Machine) with 30% O2:70% N2. The animals' femoral arteries and veins were placed with catheters including a Swan-Ganz for hemodynamic monitoring and resuscitation.
- Hemodynamic monitoring included arterial blood pressure (BP), and mean arterial blood pressure (MAP), pulmonary artery pressure (PAP), central venous pressure (CVP) and continuous CO.
- The NICOM system (Cheetah Medical) requires placement of four double electrode stickers on the thorax.
- Animals were stabilized for 15 min then hemorrhaged (controlled arterial bleed) following a stepwise hemorrhagic shock model for sheep: bleeding 35~50% total blood volume, MAP <33 mmHg for 60 minutes (class III shock model). The first fast bleeding phase: 3ml/kg/min until MAP <45 mmHg; the second bleeding phase: 2 ml/kg/min until MAP <40 mmHg starting at MAP recovered to 55 mmHg or 15 minutes after the first fast bleeding. The third bleeding phase: 1 ml/kg/min until MAP <30 mmHg (±3mmHg) starting at MAP recovered to 45 mmHg or 15 minutes after the second bleeding.
- Resuscitation was first intravenously infused hetastarch (6% hydroxyethyl starch 130/0.4 in 0.9% sodium chloride), equal volume to blood loss. Animals were stabilized for 10 minutes and maintained MAP >55 mmHg before infusing PFC or normal saline (5 ml/kg) over 15 minutes.
- Animals were observed for 60 minutes after resuscitation completed, then allowed to recover from anesthesia and returned to DAR facility. The data collected included CO and mean arterial pressure (MAP) every 20 minutes from the acutely hemorrhaged sheep from baseline, to shock, then 1 hour post-resuscitation. Data were compared between measures and over time.

Experimental Timeline





Results

- MAP at baseline averaged 68 mmHg; average baseline CO was 2.0 and 2.9 L/min for PAC and NICOM respectively.
- NICOM CO measurements were consistently higher than PAC (0.85 L/min; SE 0.16 L/min; p<0.0001; limits of agreement -1.6, 3.3 L/min). The greatest differences between methods occurred at the end of the shock period, coinciding with the greatest variability in hemodynamic stability (MAP standard deviation 23 mmHg).
- The overall correlation between PAC and NICOM measurements over the entire shock-resuscitation cycle was moderate (r = 0.65).
- Although 14/20 animals showed good to excellent congruence between methods (cross correlation r > 0.89 at zero lag), PAC readings for 6/20 sheep consistently lagged behind NICOM.
- NICOM readings had higher intrinsic variability (NICOM = 0.98; versus PAC = 0.13), however PAC readings were less reliable with 275 dropped or out-of-range observations (CO << 0.1 L/min) during the shock phase, versus none with NICOM.

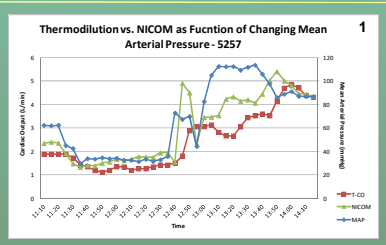


Figure 1: Total blood loss 374 mL (24.2%); resuscitated with 300 mL hetastarch and 124.5 mL treatment (saline or PFC). Total experimental period of 3 hr 5 min.

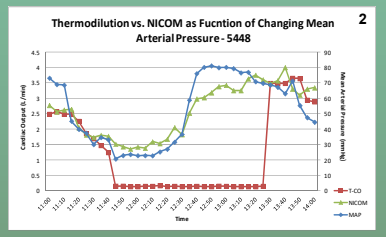


Figure 2: Total blood loss 586 mL (39.9%); resuscitated with 180 mL hetastarch and 118.5 mL treatment (saline or PFC). Total experimental period of 2 hr 50 min.

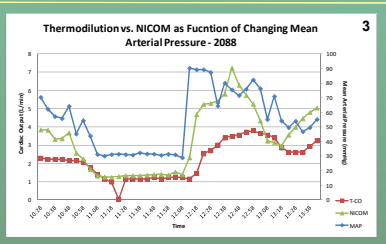


Figure 3: Total blood loss 463 mL (39.9%); resuscitated with 270 mL hetastarch and 93.5 mL treatment (saline or PFC). Total experimental period of 3 hr 15 min.

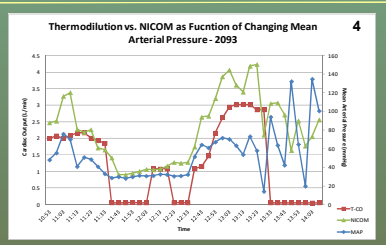


Figure 4: Total blood loss 603 mL (47.7%); resuscitated with 300 mL hetastarch and 102.0 mL treatment (saline or PFC). Total experimental period of 3 hr 15 min.

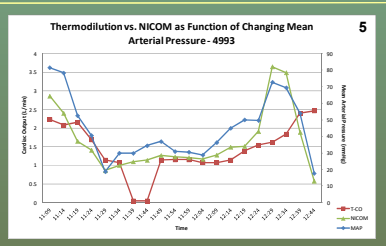


Figure 5: Total blood loss 688 mL (53.3%); resuscitated with 600 mL hetastarch and 60 mL treatment (saline or PFC). Total experimental period of 1 hr 35 min. Animal euthanized on table.

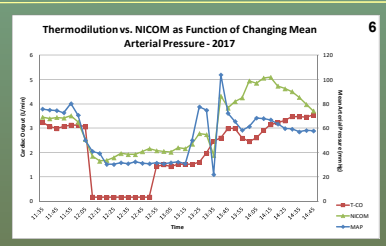
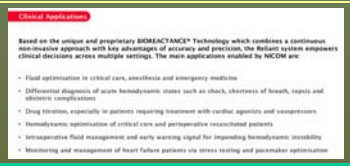
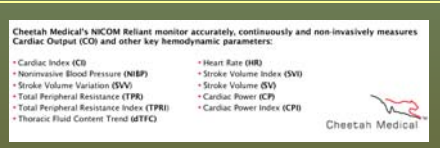


Figure 6: Total blood loss 523 mL (38.9%); resuscitated with 415 mL hetastarch and 108.5 mL treatment (saline or PFC). Total experimental period of 2 hr 50 min.





Conclusion:

- PAC was outperformed by NICOM when measuring CO in hemodynamically unstable subjects with rapidly changing MAP.
- PAC suffered from too many dropped or out-of-range readings, and generally lagged behind NICOM.
- NICOM responses were consistently reliable with acceptable accuracy in a clinically realistic model of a hemodynamically challenging situation. Availability of such a tool will allow clinicians to have information about CO in patient when the T-CO method is not feasible to help diagnose and guide therapy.
- PAC remains a viable option when left atrial pressure, pulmonary artery pressure, and central venous samples would make a significant difference in the management of a patient.

Acknowledgements:

- The Microscopy Core Facility of VCU
- The work is funded by U.S. Army Medical Research and Materiel Command (W81XWH-13-1-0017)
- PI: Dr. Bruce Spiess

Temporal Analysis Of Biomarkers Of Brain Damage In Ovine Survival Models Of Hemorrhage And Blast / Hemorrhage Polytrauma With Perfluorocarbon Treatment.

J. Travis Parsons, Suneel K. Thummala, Jacquelyn R. McCarter, Christopher R. Sweeney, Paul A. Middleton, Jiepei Zhu, Bruce D. Spiess. Virginia Commonwealth University, Richmond, VA

Background: Secondary blast injury (due to airborne shrapnel) can result in severe hemorrhage in the far forward battlefield and can be life threatening with the absence of blood products and significant delay from tactical combat casualty care to forward surgical teams. Perfluorocarbon (PFC) oxygen therapeutics are capable of effectively oxygenating sensitive tissue in the absence of adequate hemoglobin and/or blood flow. PFC emulsion volumes required for efficacy can be readily carried in a medic pack and can be easily administered by minimally trained personnel. Alpha II spectrin breakdown products (markers of neuronal necrosis and apoptosis) and S100B (marker of blood brain barrier breakdown) can be measured in plasma and can be associated with outcomes and efficacy of PFC therapy. PFC may improve the "golden hour" during en route care of far forward battlefield polytrauma soldiers.
Methods: Survival ovine (male, juvenile, 25-30 kg) models of hemorrhage and blast/hemorrhage polytrauma were used. Animals were subjected to controlled moderate stepwise hemorrhagic shock or exposed to blast overpressure (~10-15psi) utilizing an Advanced Blast Simulator just prior to hemorrhagic shock. Mean arterial blood pressure (MAP) was maintained at ~30mmHg for 60 minutes. Sheep were then resuscitated with Hespan until MAP was 65 mmHg for 10 minutes then randomized to receive intravenous PFC or saline (both 5 ml/kg). Venous plasma was collected 2 days before injury (baseline) and at 1, 4, and 7 days post injury. Plasma samples proteins were balanced by UV-Visible spectrophotometry, resolved by SDS-PAGE, and transferred to nitrocellulose. Western blots were probed for Alpha-II spectrin breakdown products or S100B using a commercially available antibody, visualized by chemiluminescence, and densitometerically analyzed. Experimental groups (all n=8): Hemorrhage+saline, Hemorrhage+PFC, polytrauma+saline, polytrauma+PFC, and controls.
Results: Total blood loss was 32.3-52.0% (Hemorrhage+saline), 29.6-48.0% (Hemorrhage+PFC), 7.4-45.7% (polytrauma+saline), and 15.2-51.6% (polytrauma+PFC). Lactate levels went from baseline to maximal mmol/L of 0.6-2.5 (Hemorrhage+saline), 0.8-2.4 (Hemorrhage+PFC), 1.5-2.2 (polytrauma+saline), and 0.5-1.4 (polytrauma+PFC). Biomarkers of neuronal cell death, alpha II spectrin breakdown products, were not observed in any of the groups at any of the time points analyzed. Likewise, biomarker of blood brain barrier disruption, S100B, was not observed in any of the groups at any of the time points analyzed.
Conclusion: Alpha II spectrin biomarkers of neuronal cell death were not observed in the plasma of sheep subjected to hemorrhagic shock or blast / hemorrhagic polytrauma. Blood brain barrier remained intact for all injury groups as no S100B was observed in plasma. It is possible that alpha II spectrin breakdown products were generated, however, since the blood brain barrier remained intact, the breakdown products would only be found in brain tissue. It is also feasible that the level of hemorrhage and polytrauma for these studies was not severe enough, as lactate levels were not greatly increased. Efficacy of PFC as a treatment modality cannot be assessed from this study as changes in biomarkers were not observed. (PI: Dr. Bruce D. Spiess; USAMRMC: W81XWH-13-1-0017)

Submitted for consideration for poster presentation for 2016 Military Health Systems Reseach Symposium.

Abstract ID: MHSRS-16-1514
Research Topic: En Route Care

The Effect of Adjunctive Perfluorocarbon Infusion on Platelet Number and Function in a Blast / Hemorrhage Polytrauma Sheep Model

Jiepei Zhu[1], J. Travis Parsons[2], Erika J. Martin[3], Jacquelyn R. McCarter[2], Christopher R. Sweeney[1], Paul A. Middleton[1], Suneel K. Thummala[1], Brian E. Berger[1], Bassem M. Mohammed[3], Donald F. Brophy[3] and Bruce D. Spiess[1] Departments of Anesthesiology[1], Neurosurgery[2], and Pharmacotherapy & Outcomes Science[3], Virginia Commonwealth University, Richmond VA 23298-0695. ***Background.*** Perfluorocarbon (PFC) emulsions can be used as an adjuvant by enhancement of oxygen delivery for trauma, hemorrhagic injury and en route care. A possible side effect of PFC is thrombocytopenia (in 30~50%) on treatment days 2-5. It is necessary to investigate this phenomenon to exclude platelet inflammatory/embolic risks before clinical trials resume. ***Methods*.** A survival sheep polytrauma model was used, which subjects the animal to blast overpressure (15 psi) utilizing an Advanced Blast Simulator followed by controlled moderate stepwise hemorrhagic shock. Animals lost 33-50% total blood volume with the mean arterial blood pressure (MAP) maintained at 30±3 mmHg for 60 minutes (class III model). A total of 20 injured sheep (juvenile, 25-30 kg) were randomly resuscitated with Hespan until MAP rose to 65 mmHg for 10 minutes then intravenous infusion PFC (Perftoran, 5 ml/kg, n=10) or saline (5 ml/kg, n=10). Venous blood was collected at 2 days before the polytrauma as a baseline measurement and at 1, 24, 96 and 168 hours post-polytraumatic injury and resuscitation. Blood samples were measured for platelet count (Plts), the mean platelet volume (MPV), platelet distribution width (PDW), fibrinogen level, von Willebrand factor antigen (vWF:Ag) and platelet function assays including platelet aggregation by collagen (Collagen AGG) and ADP (ADP AGG). Platelet activation morphology was also observed by scanning electron microscopy (SEM). ***Results.*** Plts were significantly reduced after injury and resuscitation with non-blood fluid at 1, 24 and 96 hours compared with its baseline (BL). Plts: 767±209 (BL, median±SD, 1000/μl) vs 239±87 at 24 hrs in PFC group. Similarly, Plts were reduced in saline group compared to BL (572±199 vs 191±138). Plt number returned to baseline level at 168 hours (7 days) post resuscitation compared with their baseline. There was no significant change in platelet number between groups. MPV, PDW, fibrinogen level, vWF:Ag, Collagen AGG and ADP AGG did not show a significant difference among time points and between groups. There were no significant differences between groups with respect to platelet activation morphology. ***Conclusion***. PFC intravenous treatment for polytraumatic injury did not further decrease Plts nor significantly change their level of activation compared with the control group. These data suggest intravenous PFC infusion with Perftoran does not cause coagulopathy in this animal traumatic shock model. (PI: Dr. Bruce D. Spiess; USAMRMC: W81XWH-13-1-0017)

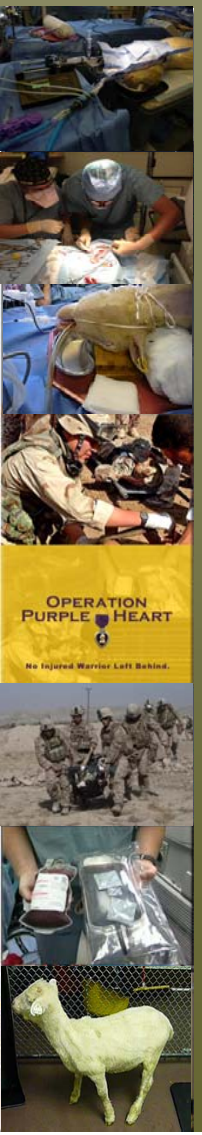
Submitted on April 7, 2016: Abstract ID: MHSRS-16-1009, Poster Presentation;
Research Topic: Hemorrhage Control & Resuscitation
Learning Objectives: What should the attendee learn from this presentation:
Use action verbs such as Describe, Define, Analyze to begin the description of each learning objective. A learning objective is one sentence.
1. Describe a sheep survival polytrauma model combined over pressure blast with hemorrhagic shock
2. Infuse perfluorocarbon as an adjunctive agent for resuscitation
3. Analyze the effect of perfluorocarbon infusion on circulatory platelet number and activation

The Effect of Adjunctive Perfluorocarbon Infusion on Platelet Number and Function in a Blast / Hemorrhage Polytrauma Sheep Model

Jiepei Zhu[1], J. Travis. Parsons[2], Erika J. Martin[3], Jacquelyn McCarter[2], Christopher Sweeney[1], Paul Middleton[1], Suneel Thummala[1], Brian Berger[1], Bassem Mohammed[3], Donald Brophy[3] and Bruce D. Spiess[4]

[1]Departments of Anesthesiology, [2]Neurosurgery, [3]Pharmacology, School of Medicine, Virginia Commonwealth University, Richmond, VA 23298-0695; [4]College of Medicine, University of Florida

MHSRS-16-1009



Introduction

Perfluorocarbon (PFC) is a non-polar oil/emulsion with enhanced respiratory gas (O_2, N_2, CO_2) solubility found in 1966. All O_2 dissolved in PFC is available for metabolic use, which is called an O_2 carrier. PFC particles are 0.1~0.2 μm and get into tissues where RBCs cannot after injury. PFC as an extra compartment for O_2 transport and has a unique efficacy in low flow states. PFC has shown efficacy in models (some human data) of hemorrhagic shock, traumatic brain injury (TBI), spinal cord injury, decompression sickness (DCS), arterial/venous gas embolism (A/VGE). Therefore, the intravenous infusion of PFC emulsions may be used as an adjuvant by enhanced oxygen delivery for trauma hemorrhagic injury and en route care in the battlefield.

A possible side effect of PFC might be related with thrombocytopenia (in 30~50%) on days 2~5 after intravenous infusion. It is necessary to investigate this phenomenon to exclude platelet inflammatory/embolic safety risks before clinical trials resume. Using a sheep blast / hemorrhagic shock model to investigate the effect of PFC on sheep platelet number and function.

Materials & Methods

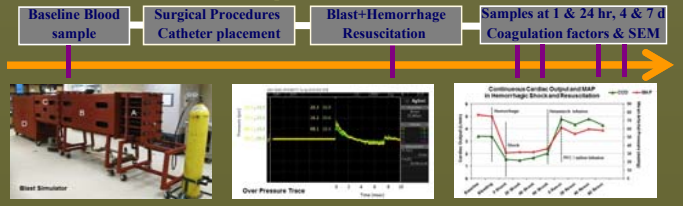
Subjects and Groups:

- The experimental protocol was reviewed and approved by the Animal Care and Use Committee of Virginia Commonwealth University (VCU) and all experiments were conducted in VCU.
- Total 30 Juvenile Dorset or Dorper sheep (18-32 kg body weight) were survived through the study. Animals were given 7 days for acclimation prior to experiment, and daily vital signs are monitored including temperature, heart rate and respiratory rate. 20 sheep blasted first then followed hemorrhagic shock were randomly resuscitated with hespan plus PFC (Perftoran) or resuscitated with hespan plus saline (5ml/kg). 10 sheep were used for surgical sham control

Blast + Hemorrhagic Shock and Resuscitation Procedures & Blood Sample Collection:

- Animal was induction of anesthesia with 5% isoflurane and maintained with 1~2% isoflurane during the procedure.
- Animal was intubated (7.5 F) and ventilated (Drager Fabius GS Anesthesia Machine) with 30% O_2: 70% N_2)
- Animal's femoral arteries and veins were placed with catheters including a swan ganz for hemodynamic monitoring and resuscitation. Hemodynamic monitoring include arterial blood pressure (BP) and mean arterial blood pressure (MAP); Central venous blood pressure (CVP); Pulmonary arterial blood pressure (PAP); Cardiac output and arterial, venous blood gases.
- Animals were subjected to blast overpressure (15 psi) utilizing an Advanced Blast Simulator followed by controlled moderate stepwise hemorrhagic shock. Animal lost 33~50% total blood volume with mean arterial pressure (MAP)=30 mmHg for 60 minutes (class III shock model). The first fast bleeding phase: 3ml/kg/min till MAP=45 mmHg; The second bleeding phase: 2 ml/kg/min till MAP =40 mmHg starting at MAP recovered to 55 mmHg or 15 minutes after the first fast bleeding. The third bleeding phase: 1 ml/kg/min till MAP =30 mmHg (±3mmHg) starting at MAP recovered to 45 mmHg or 15 minutes after the second bleeding.
- Resuscitation was first intravenously infusion hespan (6% hetastarch) till MAP = 65 mmHg. Then, animal was stabilized for 10 minutes and maintain MAP =55 mmHg before added PFC or saline (5 ml/kg) infusion in 15 minutes. Animal was observed for 60 minutes after resuscitation completed. Then, animal was allowed to recover from anesthesia and carried back to DAR facility.
- Venous blood was collected via jugular vein puncture at baseline, 1 & 24 hour and 4 & 7 day post shock.

Experimental timeline

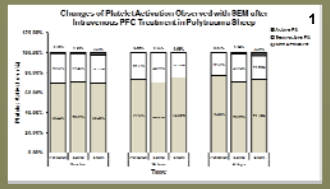


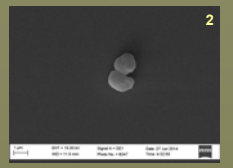
Venous blood sample measurement & Data analysis:

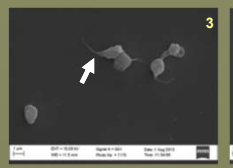
Platelet number, MPV, PDW measured with VetScan HM5 Hematology system;
Fibrinogen measured with Diagnostica Stago analyzer.
Collagen Aggregation, ADP aggregation measured with 700 Aggregometer.
Platelet CD62p measured with flow cytometry platelet activation

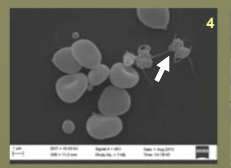
Results

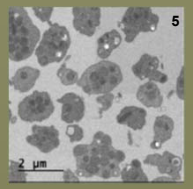
Observation of Platelet Activation with scanning electron microscopy (SEM) and transmission electron microscopy (TEM)





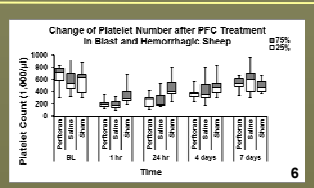


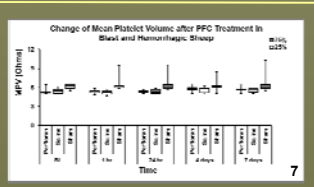


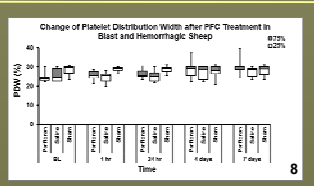


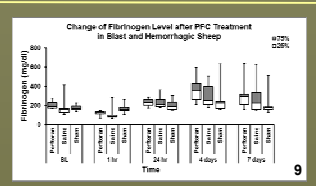
Criteria Used to Analyze Platelet Count Images

Figure 1. Summary of the percentage distribution of non-active, semi-active and active platelets .
Figure 2: Non-active platelets: Non-active platelets are small size with smooth surface. **Figure 3**: Semi-active platelets are with one or 2 processes.
Figure 4: Active platelets are with 3 or more processes (Pseudopods) and their surface becomes irregular or granular. Or conjugated platelets which groups of platelets that have pseudopods connected
Figure 5. Platelet image observed with Transmission Electronic Microscopy. Activation of platelet with: a) Swollen open canalicular system; b) Spheroid forms with pseudopodia; c) Aggregation/clumping

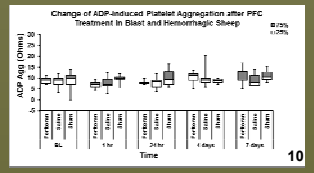


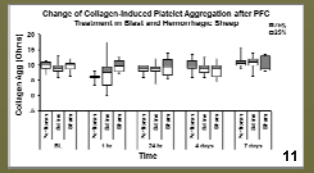


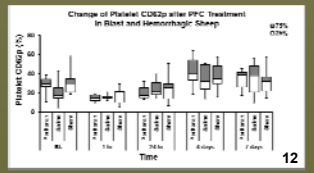


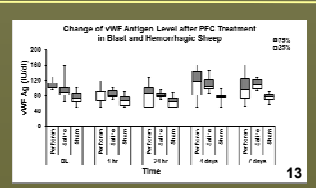


Platelet number (**Fig 6**): In normal sheep, platelet mean value is about 400,000/μl (range from 100,000 to 800,000). The results showed that platelet counts were significantly decreased at 24 hour after PFC or saline resuscitation compared with the sham group or with their own baselines. However, there were no significant changes between PFC and saline groups at any time points before or post resuscitation. Platelet number returned back to baseline level at 7 days pos-injury. Platelet count and mean distribution with standard error. Box plot shows platelet count distribution with minimum value, maximum value, 25%, median, and 75% values. **Mean platelet volume** (**Fig 7**) **and Platelet distribution width** (**Fig 8**) did not show significant changes among groups, which indicated that the decrease platelet number might be the result of non-blood fluid resuscitation (hemodilution). **Fibrinogen measurement** (**Fig 9**) showed a decrease at one hour in injured groups (PFC and Saline groups and returned to baseline level at 24 hours. There is no significant difference when the groups are compared each other at 24 hours and 4 & 7 days indicating no massive clot formation after injury and treated with PFC.









Platelet function tests: Platelet aggregation testing measures the ability of various agonists to platelets to induce in vitro activation and platelet-to-platelet activation. **ADP induced platelet aggregation (weak agonist, ADP-Agg,** Fig 10) and **Collagen induced platelet aggregation (strong agonist, Collagen-Agg,** Fig 11): the data showed that ADP-Agg and Collagen-Agg were significantly decreased at 1 hour after resuscitation and returned back to baseline level at 24 hours post-resuscitation. There were no significant changes between PFC and saline groups at any time points before or post resuscitation indicating that PFC treatment after trauma/hemorrhage did not make platelet function worse in the current injury model. Box plot shows measurements with minimum value, maximum value, 25%, median, and 75% values. **Platelet CD62p (platelet surface expression of CD62P (P-selectin),** Fig 12) was significantly decreased at 1 hour and did not show significant changes between PFC and saline groups. Platelet CD62p expression returned to baseline and increased expression at 4 days and 7 days at post-resuscitation compared with baseline. **von Willebrand Factor Antigen (Factor VIII:R Antigen,** Fig 13) is important for platelet-platelet and platelet-vessel hemostatic interactions. The data showed that vWF Ag decreased at one hour in injured groups (PFC and Saline groups) and returned to baseline level at 24 hours. There is no significant difference when the groups were compared each other at 24 hours and 4 & 7 days. Platelet function assessment results indicate that intravenous PFC treatment did not significantly change platelet function using combined blast and hemorrhagic shock sheep model.

Conclusion:

- **Intravenous infusion Perftoran (PFC) as an adjuvant resuscitation fluid does not significantly change in platelet number and functional activation comparing with saline control group in a combined blast trauma and hemorrhagic shock model in sheep.**
- **The result of quantitative observation of platelet activation with SEM is corresponded with the results of platelet function tests.**
- **Therefore, intravenous infusion with Perftoran will not significantly worse the coagulopathy caused by a moderate trauma injury.**

Acknowledgements:

- **The Microscopy Core Facility of VCU**
- **The work is funded by U.S. Army Medical Research and Materiel Command (W81XWH-13-1-0017)**
- **PI: Bruce Spiess, MD.**

Intracerebral hematoma incidence in a coagulopathic sheep model of deep brain stimulation (DBS) surgery: microelectrode versus DBS electrode penetration - Submitted: 3/18/2015

General Information

Abstract Title:	Intracerebral hematoma incidence in a coagulopathic sheep model of deep brain stimulation (DBS) surgery: microelectrode versus DBS electrode penetration
Study Design:	Laboratory Investigation
Preferred Format:	Any Format
Subject Category:	Stereotactic/Functional
Award Consideration:	Stereotactic and Functional Neurosurgery Resident Award

Scientific Content

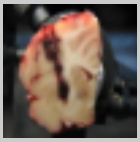
Introduction:	Intracerebral hemorrhage is an infrequent (cited as 0.9-5%) but often devastating complication of deep brain stimulation (DBS) surgery. Observational human studies have suggested that the use of microelectrode (ME) for recording is correlated with a higher risk of intraoperative hemorrhage, but this remains highly controversial. The goal of our experimental study was to directly compare brain hemorrhage rates when using DBS electrodes versus ME in a coagulopathic sheep model of DBS surgery.
Methods:	Healthy male sheep underwent bilateral strip craniectomies followed by IV bolus of 100 U/kg heparin. A modified NexFrame device was used to insert hardware in 5 sheep: MEs on one side of the brain and DBS electrodes on the other. The hardware was inserted 20 mm into the brain parenchyma through all five Bengun channels. This was done at 4 sites per hemisphere resulting in total 100 of DBS and 100 of ME tracks available for study. The ME and DBS insertions were performed simultaneously to maintain similar conditions at the time of insertion. Harvested brains were sectioned in a cryostat and evaluated in blinded fashion for the presence of deep (> 5 mm depth) hematomas.
Results:	Deep hematomas were detected in 16% of DBS tracks and in 12% of ME tracks (p = 0.504). The average hematoma volume was 3 times larger in DBS tracks (80.8 ± 31.1 mm3; 95% CI) than in ME tracks (29.4 ± 22.5 mm3; 95% CI).
Conclusions:	We designed a sheep brain hemorrhage model utilizing standard human hardware to provide a comparatively high rate of intraparenchymal bleeding. Our results did not demonstrate a higher risk of bleeding with the use of microelectrodes as compared to DBS electrodes. We plan to continue this work to analyze other factors, such as various hardware properties and insertion techniques, which may influence the incidence of intracerebral hemorrhage.

Learning Objectives:	By the conclusion of this session, participants should be able to: 1) Describe the importance of DBS hardware features at inducing intracerebral hematomas, 2) Discuss, in small groups, the incidence and extent of hemorrhagic complications in DBS surgery 3) Identify a safe DBS electrode placement techniques.
Keywords:	Animals, sheep, heparin, coagulopathy, intracerebral, hematoma, incidence, electrodes, microelectrodes, deep brain stimulation
How will your research improve patient care:	Will minimize the risk of intracerebral hemorrhage during DBS surgery.

Attachments:

Title: Microelectrode tracks in basal ganglia
Caption: Microelectrode tracks in basal ganglia

Title:DBS electrode tracks in basal ganglia
Caption:DBS electrode tracks in basal ganglia



Title: Deep and superficial intracerebral hematomas
Caption: Deep and superficial intracerebral hematomas

Title: Superficial intracerebral hematoma
Caption: Superficial intracerebral hematoma

Authors:

Author	Disclosure	Presenting	Corresponding	CoAuthor
Viktoras Palys		X	X	
Daniel Lotz				X
Mike Waters				X
Jiepei Zhu				X

Kathryn Holloway	Medtronic,,Other,N/A St Jude,,Other,N/A	X
Travis Parsons		X

Effect of Perfluorocarbon on Platelet Number and Function after Intravenous Infusion in Sheep

Jiepei Zhu[1], J. Travis Parsons[2], Jacquelyn R. McCarter[2], Christopher R. Sweeney[1], Jackson M. Hylton[1], Erika J. Martin[3], Donald Brophy[3] and Bruce D. Spiess[1]
Departments of Anesthesiology[1], Neurosurgery[2], and Pharmacology[3], Virginia Commonwealth University, Richmond VA 23298-0695. ***Background.*** Perfluorocarbon emulsions (PFC) can treat traumatic injuries in the battlefield by enhanced delivery of oxygen. A possible side effect of PFC may be thrombocytopenia (in 30~50%) on days 2~5 after intravenous treatment. It is necessary to investigate this phenomenon to exclude platelet inflammatory/embolic safety risks before clinical trial. ***Methods***. Total 24 healthy juvenile sheep (25-30 kg) were randomly divided into 3 groups (n=8/group) with a top load intravenous infusion with either PFC (Oxygent, 60%, 3 g/kg), Hespan (6% hetastarch), or naïve/saline control (naïve =4, saline=4). Venous blood was sampled before the treatment (baseline) and at 0 minute, 3 and 24 hours, 4 and 7 days after infusion and were measured for platelet count, fibrinogen, clot formation time, ADP aggregation & CD62p, etc. Platelet activation was quantitatively observed with scanning electron microscopy (SEM). ***Results.*** Comparing baseline with other time points, there were no significant differences on platelet count among control, PFC and Hespan group (435.92±89.42; 391.15±46.60; 437.16±33.63; unit 1000/dl, mean±SE at 4day post infusion); and fibrinogen level (197.00±20.59; 291.38±79.36; 218.13±25.95; unit mg/dl at 4 day post infusion). Clot time, clot forming time and platelet activation assay (CD62p, %) were not increased compared with baseline or among groups. Morphologically, semi or full-activated platelets (%) were not significantly changed among groups ($p>0.05$). ***Conclusion***. Intravenous infusion with Oxygent in healthy sheep did not cause significant reduction in number of platelets nor change their activation. Therefore, intravenous infusion with Oxygent will not cause massive or severe coagulopathy. This work was supported by U.S. Army Medical Research and Materiel Command (W81XWH-13-1-0017)

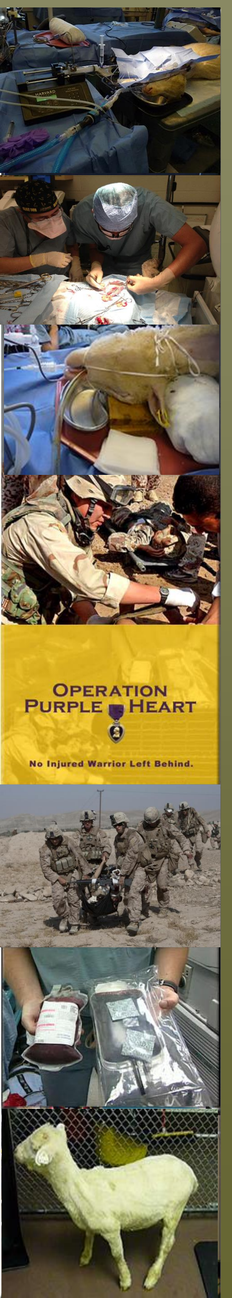
Abstract for Military Health System Research Symposium (MHSRS)
https://mhsrs.amedd.army.mil (register is needed)

2014 Abstract Submission (Due on April 4, 2014 at 5:00 PM ET, notifications will be sent Mid-May 2014)
Abstracts must be no more than 300 words (2000 characters including spaces) and contain a Background, Methods, Results and Conclusion section.

Effect of Perfluorocarbon on Platelet Number and Function after Intravenous Infusion in Sheep

Jiepei Zhu[1,4], J. Travis. Parsons[2,4], Ph.D., Jacquelyn McCarter[2], Christopher Sweeney[1], J. Mark Hylton, Jr. [1], Erika J. Martin[3,4], Donald Brophy[3,4] and Bruce D. Spiess[1,4],

[1]Departments of Anesthesiology, [2]Neurosurgery, [3]Pharmacology and [4]VCURES- Virginia Commonwealth University Reanimation Engineering Shock Center, School of Medicine, Virginia Commonwealth University, Richmond, VA 23298-0695



Introduction

- PFC is a non-polar oil/emulsion with enhanced respiratory gas (O_2, N_2, CO_2) solubility found in 1966.
- All O_2 dissolved in PFC is available for metabolic use, which is called an O_2 carrier.
- PFC particles are 0.1~0.2 µm and get into tissues where RBCs cannot after injury.
- PFC as an extra compartment for O_2 transport and has a unique efficacy in low flow states.
- PFC shows efficacy in models (some human data) of hemorrhagic shock, traumatic brain injury (TBI), spinal cord injury, decompression sickness (DCS), arterial/venous gas embolism (A/VGE), stroke, etc..
- 9 TBI patients were treated with PFC in MCVH with good outcome (Drs. Spiess/Bullock, 2006) .
- PFC may be related with thrombocytopenia (in 30~50%) on days 2~5 after intravenous infusion.
- FDA requests investigation of the phenomenon to exclude platelet inflammatory / embolic safety risks.
- Using a healthy sheep top-loaded (PFC) model and a combined hemorrhagic shock blast traumatic brain injury model to investigate the changes of platelet number and function.

Materials & Methods

Subjects and Groups:

- The experimental protocol was reviewed and approved by the Animal Care and Use Committee of Virginia Commonwealth University
- Total 24 Juvenile Dorset or Dorper sheep (18-32 kg body weight) were used and randomly divided into 3 groups
- PFC group (n=8); Hespan group (n=8); and Control group (n=8; naïve n=4; and saline n = 4)
- Animals were given 7 days for acclimation prior to experiment, and daily vital signs are monitored including temperature, heart rate and respiratory rate.

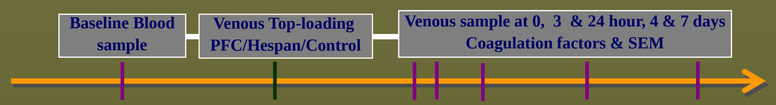
Top-load Procedure & Blood Sample Collection:

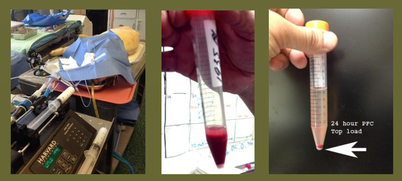
- Animal was induction of anesthesia with 5% isoflurane and maintained with 2% isoflurane during the procedure.
- Animal was intubated (7.5 F) and ventilated (Drager Fabius GS Anesthesia Machine) with 30% O_2; 70% N_2)
- Animal's neck fur was shaved and sanitized; 20 G x 2' needle catheter was puncture into external jugular vein
- PFC (Oxygent, 60%) or Hespan (6% Hetastarch) was intravenous infusion with 5 ml/kg over 15 minutes.
- Animal was allowed to recover from anesthesia and carried back to DAR facility after Top-load completed.
- Venous blood was collected via jugular vein puncture at baseline, 0 min, 3 & 24 hour and 4 & 7 day post top-load.

Blood sample measurement & Data analysis:

- Venous samples were measured for coagulatory factors including:
  - Platelet number count and Alanine aminotransferase (ALT) were measured by VetScan HM5 Hematology system.
  - Fibrinogen was measured using STA Fibrinogen reagent on Diagnostica Stago analyzer.
  - Clotting time, Clot formation time and Clot Angle were measured with Rotem® *delta* (Native, Intrinsic, Extrinsic).
  - Collagen Aggregation, ADP aggregation were measured with 700 Aggregometer.
  - CD62p and vWF Ag (von Willebrand Factor antigen) were measured by Elisa.
- Venous samples collected at baseline, 24 hour and 4 days post top-load were processed for morphological observation using. scanning electron microscope.
- All data was analysis using JMP 11.

Experimental timeline





Left photo: Anesthetized sheep PFC was receiving PFC infusion with 5 ml/kg (3 g/kg) over 15 minutes.

Middle and Right Photos: Platelet rich plasma preparation. PFC was seen to stay in circulating blood at least for 24 hours post PFC infusion (arrow).

Results

Morphology of platelet observation with scanning electron microscopy

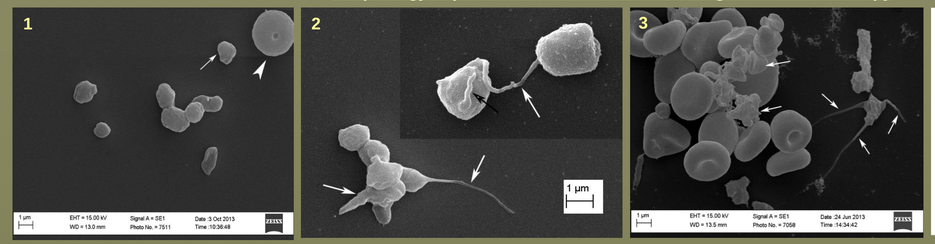
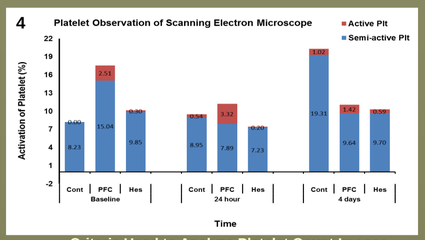
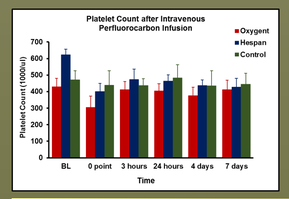


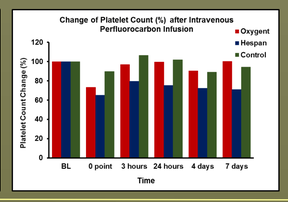
Figure 1: (left) Non-active platelets (white arrow) and red blood cell (arrow head). Non-active platelets are small size with smooth surface.
Figure 2: (middle) Semi-activate platelets are with one or 2 processes (white or black arrows) and increase their size.
Figure 3: (right) Active platelets are with 3 or more processes on surface (white or black arrows) and their surface becomes irregular or granular.
Figure 4. Percentage of active platelets and semi-active platelets. Cont =control group (n=8); PFC = Oxygent group (n=8); Hes = Hespan group (n=8). Plt = platelet. Platelets were count and calculated how many platelets were active or semi-active (see left table)

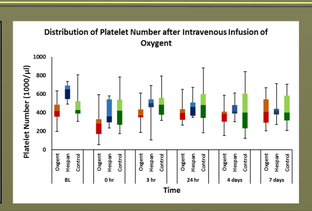


Criteria Used to Analyze Platelet Count Images

- Semi-activated platelet: with 1 or 2 pseudopods
- Full-activated platelet: 3 or more pseudopods or conjugated platelets which groups of platelets that have pseudopods connected
- Non-activated platelet: small size and smooth surface without pseudopods or processes

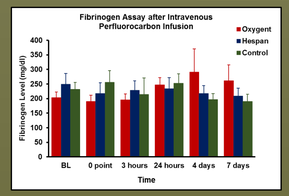


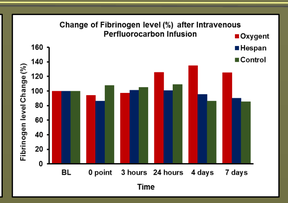


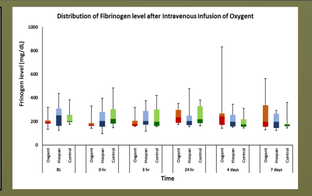


Platelet number: In normal sheep, platelet mean value is about 400,000/µl (range from 100,000 to 800,000). Our study data showed that platelet count did not change significantly when compared with control and Hespan groups (p>0.05)

Left figure: Platelet count and mean distribution with standard error
Middle figure: Percentage change of platelet count after Oxygent infusion.
Right figure: Box plot shows platelet count distribution with minimum value, maximum value, 25%, median and 75% values.

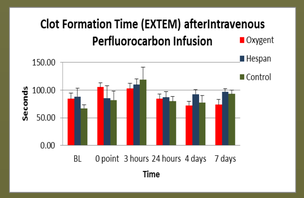




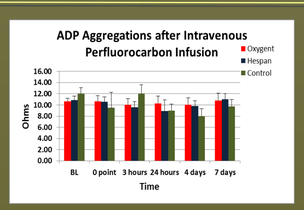


Fibrinogen measurement: There is no significant difference when the groups are compared each other after top-load with Oxygent or Hespan. Also, no significant difference was found within the groups when different time points were compared (p>0.05). Even at day 4, Oxygent group showed a higher fibrinogen measurement (one case), but there is statistically no significant difference.

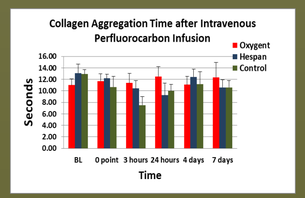
Left figure: Fibrinogen assay and mean distribution with standard error
Middle figure: Percentage change of fibrinogen after Oxygent infusion.
Right figure: Box plot shows fibrinogen level distribution with minimum value, maximum value, 25%, median and 75% values.



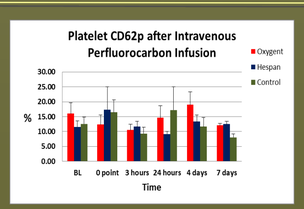
Clot Formation Time (Extem)
There is no significant difference when groups is compared after top-load with Oxygent or Hespan. Also, no significant difference was found within the groups when different time points were compared (p>0.05).



ADP Aggregations
There was no significant difference when groups were compared after top-load with Oxygent or Hespan. Also, no significant difference was found within the groups when different time points were compared (p>0.05).



Collagen Aggregation
There was no significant difference when groups were compared after top-load with Oxygent or Hespan. Also, no significant difference was found within the groups when different time points were compared (p>0.05**).**



CD62p
There was no significant difference when groups were compared after top-load with Oxygent or Hespan. Also, no significant difference was found within the groups when different time points were compared (p>0.05).

Conclusion:

- After intravenous infusion oxygent (PFC), there is no significant change in platelet number and function.
- The result of quantitative observation of platelet is corresponded with the results of coagulation factor analysis.
- Therefore, intravenous infusion with Oxygent will not cause massive or severe coagulopathy.

Acknowledgements:

- Core laboratories of Research, Department of Anesthesiology & The Microscopy Core Facility of VCU
- The work is funded by U.S. Army Medical Research and Materiel Command (W81XWH-13-1-0017 PI: Dr. Bruce Spiess)

Effect of Intravenous Perfluorocarbon on Platelet Number and Function in Hemorrhagic Sheep

Jiepei Zhu[1,4], J. Travis. Parsons[2,4], Ph.D., Erika J. Martin[3,4] , Jacquelyn McCarter[2], Christopher Sweeney[1], Paul Middleton, Donald Brophy[3,4] and Bruce D. Spiess[1,4],

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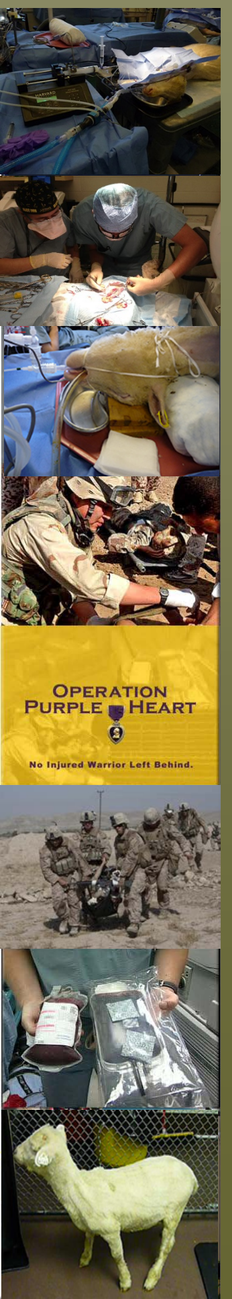
MHSRS-15-0376



Introduction

PFC is a non-polar oil/emulsion with enhanced respiratory gas (O_2, N_2, CO_2) solubility found in 1966. All O_2 dissolved in PFC is available for metabolic use, which is called an O_2 carrier. PFC particles are 0.1~0.2 µm and get into tissues where RBCs cannot after injury. PFC as an extra compartment for O_2 transport and has a unique efficacy in low flow states. PFC has shown efficacy in models (some human data) of hemorrhagic shock, traumatic brain injury (TBI), spinal cord injury, decompression sickness (DCS), arterial/venous gas embolism (A/VGE), and can be used as a part of a battlefield intravenous resuscitation fluid by enhanced oxygen delivery for trauma hemorrhagic injury and en route care.

A possible side effect of PFC might be related with thrombocytopenia (in 30~50%) on days 2~5 after intravenous infusion. It is necessary to investigate this phenomenon to exclude platelet inflammatory/embolic safety risks before clinical trials resume. Using a sheep hemorrhagic shock model to investigate the effect of PFC on sheep platelet number and function.

Materials & Methods

Subjects and Groups:

- The experimental protocol was reviewed and approved by the Animal Care and Use Committee of Virginia Commonwealth University
- Total 27Juvenile Dorset or Dorper sheep (18-32 kg body weight) were survived through the study. Animals were given 7 days for acclimation prior to experiment, and daily vital signs are monitored including temperature, heart rate and respiratory rate. 18 hemorrhagic shock sheep were randomly resuscitated with hespan plus PFC (Oxygent, 5 ml/kg, n=9) or resuscitated with hespan plus saline (5ml/kg, n=9). 9 sheep were used for surgical sham control group.

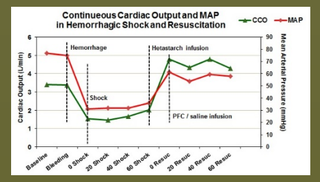
Hemorrhagic Shock and Resuscitation Procedures & Blood Sample Collection:

- Animal was induction of anesthesia with 5% isoflurane and maintained with 1~2% isoflurane during the procedure.
- Animal was intubated (7.5 F) and ventilated (Drager Fabius GS Anesthesia Machine) with 30% O_2: 70% N_2)
- Animal's femoral arteries and veins were placed with catheters including a swan ganz for hemodynamic monitoring and resuscitation. Hemodynamic monitoring include arterial blood pressure (BP) and mean arterial blood pressure (MAP); Central venous blood pressure (CVP); Pulmonary arterial blood pressure (PAP); Cardiac output and arterial, venous blood gases.
- A survival moderate stepwise hemorrhagic shock model in sheep: bleeding 35~50% total blood volume, MAP=30 mmHg for 60 minutes (class III shock model). The first fast bleeding phase: 3ml/kg/min till MAP=45 mmHg; The second bleeding phase: 2 ml/kg/min till MAP =40 mmHg starting at MAP recovered to 55 mmHg or 15 minutes after the first fast bleeding. The third bleeding phase: 1 ml/kg/min till MAP =30 mmHg (±3mmHg) starting at MAP recovered to 45 mmHg or 15 minutes after the second bleeding.
- Resuscitation was first intravenously infusion hespan (6% hetastarch) till MAP = 65 mmHg. Then, animal was stabilized for 10 minutes and maintain MAP =55 mmHg before added PFC or saline (5 ml/kg) infusion for 15 minutes. Animal was observed for 60 minutes after resuscitation completed. Then, animal was allowed to recover from anesthesia and carried back to DAR facility.
- Venous blood was collected via jugular vein puncture at baseline, 1 & 24 hour and 4 & 7 day post shock.

Experimental timeline

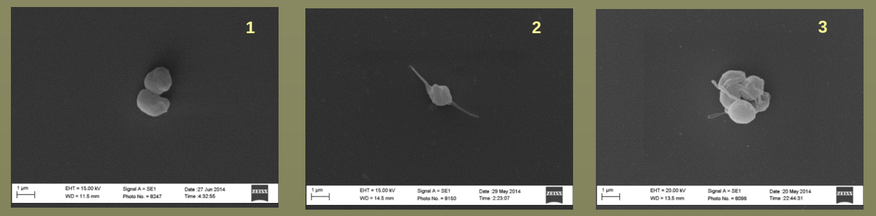
Blood sample measurement & Data analysis:

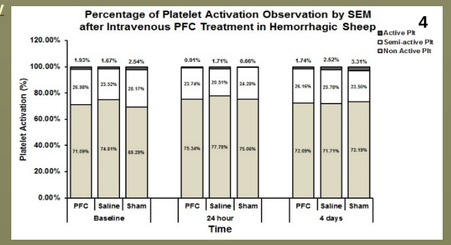
- Venous samples were measured for coagulatory factors including:
  Platelet number count (VetScan HM5 Hematology system); Fibrinogen (Diagnostica Stago analyzer).
  Platelet contractile force (PCF); Clot elastic modulus (CEM); with Rotem® *delta* (Native, Intrinsic, Extrinsic).
  Collagen Aggregation, ADP aggregation were measured with 700 Aggregometer.
- Venous samples collected at baseline, 24 hour and 4 days post shock were processed for scanning electron microscope observation.



Results

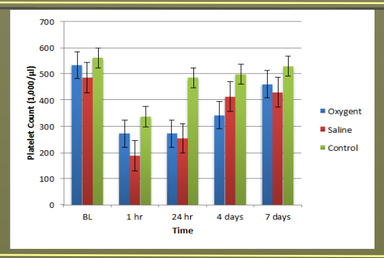
Morphology of platelet observation with scanning electron microscopy

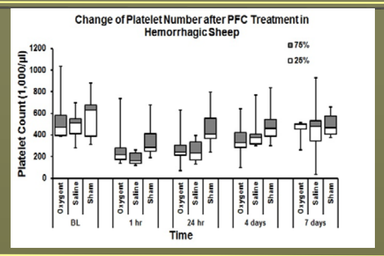




Criteria Used to Analyze Platelet Count Images

Figure 1: (left) Non-active platelets: Non-active platelets are small size with smooth surface. **Figure 2:** (middle) Semi-active platelets are with one or 2 processes.
Figure 3: (right) Active platelets are with 3 or more processes (Pseudopods) and their surface becomes irregular or granular. Or conjugated platelets which groups of platelets that have pseudopods connected
Figure 4. Percentage distribution of non-active, semi-active and active platelets .

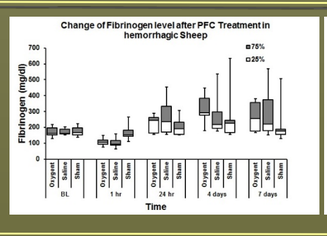


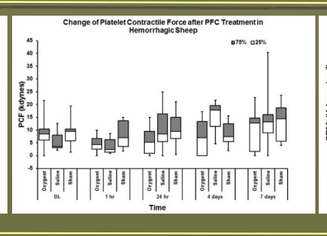


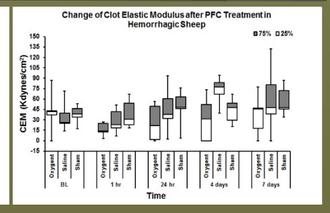
Platelet number: In normal sheep, platelet mean value is about 400,000/µl (range from 100,000 to 800,000). Our data showed that platelet count was significantly decreased at 24 hour after PFC or saline resuscitation compared with the sham procedure group or with their baseline. But there was no significant change in platelet number between PFC and saline groups at 24 hours post resuscitation. There was no significant difference in platelet count at 4 days and 7 days post shock among the groups. Platelet count returned back to baseline level at 7 days after shock.

Left Figure: Platelet count and mean distribution with standard error.

Right figure: Box plot shows platelet count distribution with minimum value, maximum value, 25%, median, and 75% values





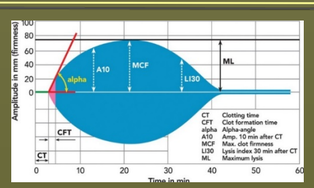


Fibrinogen measurement (left figure): analysis showed that fibrinogen dropped at one hour post resuscitation possibly because of hemodilution when comparing with sham group and baseline. There is no significant difference when the groups are compared each other at 24 hours and 4 & 7 days indicating no massive clot formation.

Platelet Contractile Force (PCF, middle figure): there is no significant difference in fibrinogen counts between the 3 groups though trend to decrease. PCF measures platelet function and is affected by platelet metabolic status, GP IIb/IIIa status, and presence of antithrombin activity.

Clot elastic modulus (CEM, on right figure) measures showed a trend decrease at 1 hour and 24 hour after PFC infusion comparing its baseline or sham. platelet function, changing with production of force by platelets, rate of thrombin generation, and platelet concentration.

PFC does not over activate or significantly reduce platelet function based on PCF & CEM measurements. Box plot figures showed the measurement distribution with minimum value, maximum value (error bar), 25%, median (between two boxes) and 75% values.



Rotational thromboelastometry (ROTEM) for hemostasis analysis

Table 1. Clotting Time (CT, seconds, mean ± SE)

Parameters	Groups	Baseline	1 hour	24 hour	4 days	7 days
Clotting Time (CT) INTEM (sec)	Oxygent	159.78±12.61	187.33±12.08	177.78±13.94	156.89±13.25	171.89±13.94
	Saline	160.44±9.01	180.22±7.87	147.78±10.86	138.78±4.72	162.00±10.86
	Sham	156.10±7.95	156.10±11.34	163.30±9.44	159.80±6.82	149.30±9.44
Clotting Time (CT) EXTEM (sec)	Oxygent	74.44±5.83	94.00±5.58	68.78±8.83	78.22±9.71	78.11±8.33
	Saline	68.44±5.22	89.00±6.09	75.78±9.24	69.89±10.00	78.00±9.24
	Sham	62.80±6.22	66.10±7.09	71.40±9.57	83.30±10.71	79.80±9.57

Clotting time comparison at baseline and various survival time points at post hemorrhagic shock. Adding PFC did not cause a significant longer clotting time.

Table 2. Maximum Clot Firmness (MCF, mm, mean ± SE)

Parameters	Groups	Baseline	1 hour	24 hour	4 days	7 days
Maximum Clot Firmness (MCF) INTEM (mm)	Oxygent	75.89±1.22	66.89±1.74	77.22±1.01	81.67±0.93	83.44±1.36
	Saline	76.00±1.09	66.78±1.26	75.44±1.39	81.33±1.42	83.44±1.39
	Sham	78.40±0.88	76.50±1.02	79.90±1.55	80.20±1.31	80.20±1.55
Maximum Clot Firmness (MCF) EXTEM (mm)	Oxygent	75.89±1.21	66.78±1.27	76.33±1.52	82.11±0.95	83.22±1.52
	Saline	76.00±1.00	66.89±2.85	72.67±1.54	81.22±1.47	83.89±1.54
	Sham	78.10±0.84	76.40±0.87	79.80±1.81	80.10±1.39	80.50±1.81

Maximum clot firmness comparison at baseline and various survival time points at post hemorrhagic shock. Adding PFC did not significantly reduce MCF.

Conclusion:

- After intravenous infusion oxygent (PFC) following shock, there is no significant change in platelet number, morphology and function comparing with saline control group.
- The result of quantitative observation of platelet is corresponded with the results of coagulation factor analysis.
- Therefore, intravenous infusion with Oxygent will not significantly worse the coagulopathy caused by hemorrhage.

Acknowledgements:

- The Microscopy Core Facility of VCU
- The work is funded by U.S. Army Medical Research and Materiel Command (W81XWH-13-1-0017)
- PI: Dr. Bruce Spiess

The Effect of Perfluorocarbon Oxygen Therapeutics in a Sheep Survival Model of Severe Hemorrhagic Shock

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Virginia Commonwealth University, Richmond, Virginia, USA

MHSRS 2015 #2041

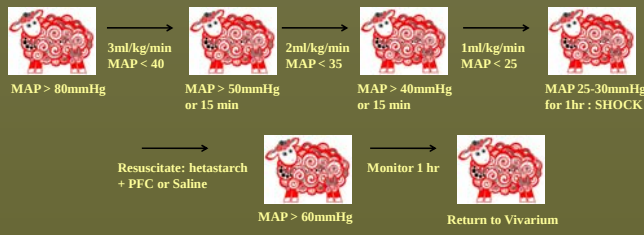
BACKGROUND

Severe hemorrhagic shock in the far forward battlefield can be life threatening with the absence of blood products and significant delay to higher echelon treatment facilities. Perfluorocarbon (PFC) oxygen therapeutics are capable of effectively oxygenating sensitive tissue in the absence of adequate hemoglobin and/or blood flow. PFC emulsion volumes required for efficacy can be readily carried in a medic pack and can be easily administered by minimally trained personnel. PFC may improve the "golden hour" during en route care of far forward battlefield polytrauma soldiers.

METHODS

Hemorrhagic Shock

Male sheep (20-30kg) were anesthetized, intubated, and ventilated on room air with 1-2% isoflurane. Animals were instrumented for measurement of vitals, hemodynamics, and sampling of blood for gases, biochemistry, and hematologic evaluation. Arterial blood was removed 3ml/kg/min until MAP below 40mmHg. Once MAP returned to 50 (or 15min), blood was removed 2ml/kg/min until MAP below 35mmHg. Once MAP returned to 40 (or 15min), blood was removed 1ml/kg/min until MAP below 25mmHg. Sheep remained at MAP 25-30mmHg for 1hr. Sheep were then resuscitated with minimal volume hetastarch (until MAP 60mmHg) then given either 6cc/kg saline (n=3) or Oxygent PFC (n=3). Following resuscitation, sheep vitals and hemodynamics were monitored for 1 hour before returning to vivarium.

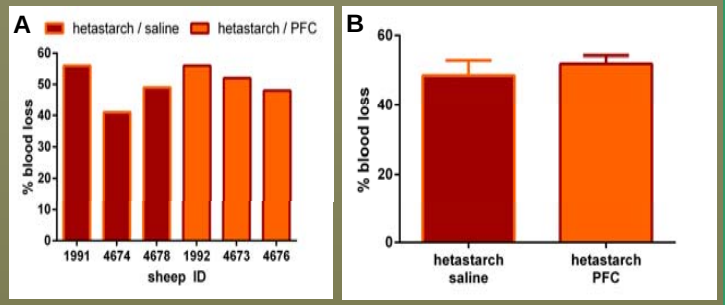


Behavioral Analyses

Efficacy of PFC treatment was evaluated by behavioral analysis using unobtrusive video camera monitoring of sheep in their social flock. Animals were considered injured if more time was required for return to ambulation and more time spent laying down than standing with the flock and eating.

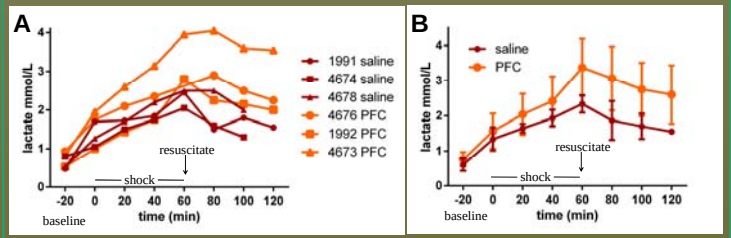
RESULTS

Total Blood Loss From Hemorrhage in (A) Individual Sheep and (B) averaged



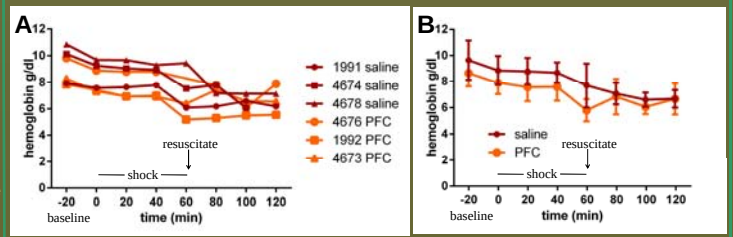
Total blood volume loss was 41-56% (48.7 ± 4.3 SEM) and 48-56% (52.0 ± 2.3 SEM) for shock saline and shock PFC treated, respectively

Lactate Levels in (A) Individual Sheep and (B) averaged



Lactate levels increased from baseline 0.60 ± 0.10 SEM and 0.76 ± 0.12 SEM mmol/L to maximal 2.33 ± 0.14 SEM and 3.38 ± 0.58 SEM mmol/L shock saline and shock PFC treated, respectively

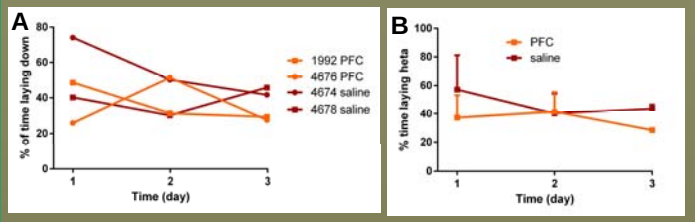
Hemoglobin Levels in (A) Individual Sheep and (B) averaged



Hemoglobin levels dropped from baseline 9.64 ± 0.87 SEM and 8.65 ± 0.60 SEM g/dL to minimal 6.61 ± 0.31 SEM and 5.80 ± 0.60 SEM g/dL shock saline and shock PFC treated, respectively.

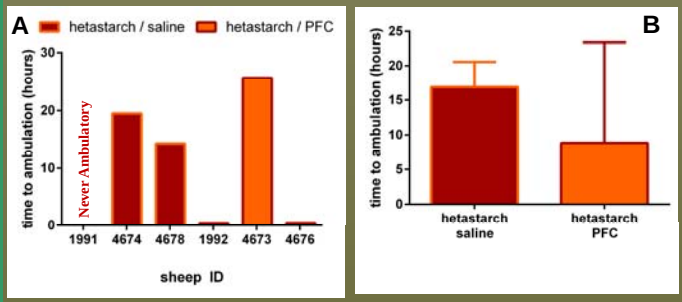
RESULTS (cont.)

Time Spent Laying Down in (A) Individual Sheep and (B) Averaged



Following hemorrhagic shock: on day one 57.20 ± 16.96% SEM vs 37.27 ± 11.26% SEM, on day two 40.25 ± 9.94% SEM vs 41.37 ± 9.92% SEM, and on day three 43.74 ± 2.03% SEM vs 28.55 ± 0.87% SEM of the time laying down, shock saline and shock PFC treated, respectively.

Time Spent Laying Down in (A) Individual Sheep and (B) Averaged



Shock saline animals took 14.3 and 19.5 hrs (16.90 ± 2.60 SEM) to ambulation and one never recovered. Shock PFC sheep took 25, 27min, and 25.6hrs (8.85 ± 8.41 SEM) to ambulation.

CONCLUSIONS

- In hemorrhagic shock animals with similar loss of blood, rise in lactate levels, and drop in hemoglobin values, the data suggest PFC treatment improves outcomes compared to animals treated with saline.
- This improvement occurs under normoxic conditions without the need for higher-than-atmospheric FiO2, not readily available in the far forward arena.
- PFC given acutely following minimal volume resuscitation after severe hemorrhage may improve outcomes for soldiers with significant evacuation timelines to higher echelon treatment facilities.

Acknowledgements: Supported by VCU Dept of Surgery Trauma Fund to JTP and U.S. Army Medical Research and Materiel Command (W81XWH-13-1-0017) to BDS

Morphological Characteristics of Platelets Post Perfluorocarbon Emulsion Infusion

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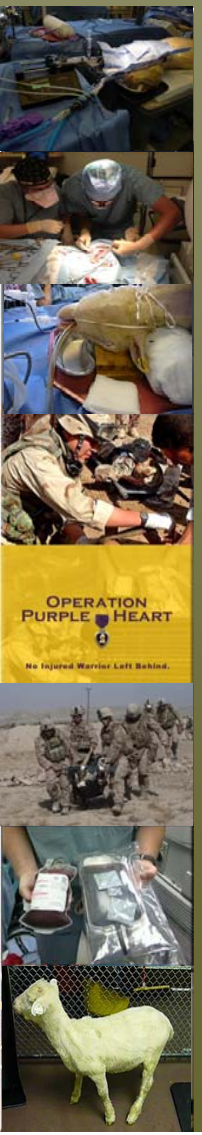
** MD candidate, Class of 2016, [1]Departments of Anesthesiology, [2]Neurosurgery, and [3]VCURES- Virginia Commonwealth University Reanimation Engineering Shock Center, School of Medicine, Virginia Commonwealth University, Richmond, VA 23298-0695*



Abstract

Background: Perfluorocarbon emulsions (PFC) can treat traumatic injuries in the battlefield by enhanced delivery of oxygen. However, a possible side effect of PFC may be thrombocytopenia (in 30~50%) on days 2~5 after intravenous treatment. It is necessary to investigate this phenomenon to exclude platelet inflammatory/embolic safety risks before clinical trial. ***Methods:*** A total 24 sheep were randomly divided into 3 groups (n=8/group) with a top load intravenous infusion with either PFC (Oxygent, 60%, 3 g/kg), Hespan (6% hetastarch), or control. Venous blood was sampled before the treatment (baseline) and 24 hours, as well as 4 days after treatment. Platelet rich plasma was isolated and quantitatively observed with scanning electron microscopy (SEM). ***Results:*** Morphologically, total platelet count, semi or full-activated platelets (%) were not significantly changed within or among groups ($p>0.05$), which is corresponding with platelet count and other coagulatory factor measurement. ***Conclusion:*** Intravenous infusion with Oxygent in healthy sheep did not cause significant reduction in number of platelets nor change their activation morphologically. Therefore, intravenous infusion with Oxygent will not cause massive or severe coagulopathy.

Introduction

- PFC is a non-polar oil/emulsion with enhanced respiratory gas (O_2, N_2, CO_2) solubility found in 1966.
- All O_2 dissolved in PFC is available for metabolic use, which is called an O_2 carrier.
- PFC particles are 0.1~0.2 μm and get into tissues where RBCs cannot after injury.
- PFC as an extra compartment for O_2 transport and has a unique efficacy in low flow states.
- PFC shows efficacy in models (some human data) of hemorrhagic shock, traumatic brain injury (TBI), spinal cord injury, decompression sickness (DCS), arterial/venous gas embolism (A/VGE), stroke, etc..
- 9 TBI patients were treated with PFC in MCVH with good outcome (Drs. Spiess/Bullock, 2006) .
- PFC may be related with thrombocytopenia (in 30~50%) on days 2~5 after intravenous infusion.
- FDA requests investigation of the phenomenon to exclude platelet inflammatory / embolic safety risks.
- Using a healthy sheep top-loaded (PFC) model and a combined hemorrhagic shock blast traumatic brain injury model to investigate the changes of platelet number and function.

Materials & Methods

Subjects and Groups:

- The experimental protocol was reviewed and approved by the Animal Care and Use Committee of Virginia Commonwealth University
- Total 24 Juvenile Dorset or Dorper sheep (18-32 kg body weight) were used and randomly divided into 3 groups
- PFC group (n=8); Hespan group (n=8); and Control group (naïve n=4; and saline n = 4)
- Animals were given 7 days for acclimation prior to experiment, and daily vital signs are monitored.

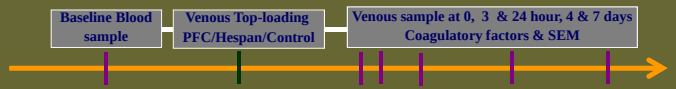
Top-load Procedure & Blood Sample Collection:

- Animal was induction of anesthesia with 5% isoflurane and maintained with 2% isoflurane.
- Animal was intubated with 7.5 F tracheal tube and ventilated (Drager Fabius GS Anesthesia Machine)
- Animal's neck fur was shaved and sanitized; 20 G x 2' needle catheter was puncture into external jugular vein
- PFC (Oxygent, 60%) or Hespan (6% Hetastarch) was intravenous infusion with 5 ml/kg over 15 minutes.
- Animal was allowed to recover from anesthesia and carried back to DAR facility after Top-load completed.
- Venous blood was collected via jugular puncture at baseline, 0 min, 3 & 24 hour and 4 & 7 day post top-load.

Blood sample measurement & Data analysis:

- Venous samples were measured for coagulatory factors including: platelet count, fibrinogen, clot formation time, ADP aggregation & CD62p, etc..
- Venous samples of baseline, 24 hour and 4 days post top-load were processed for morphological observation using scanning electron microscope.
- All data was analysis using JMP 11.0

Experimental timeline



Results

Morphology of platelet observation with scanning electron microscopy

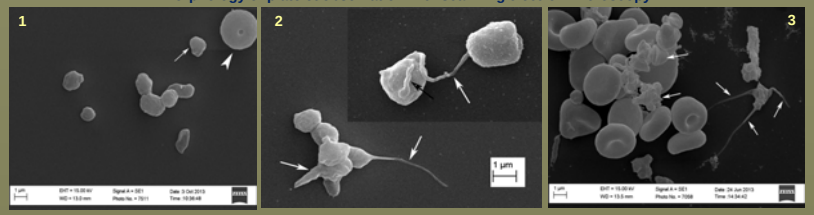
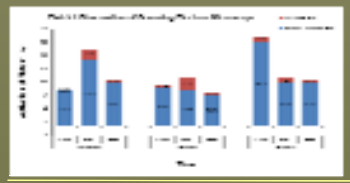


Figure 1: (left) Non-active platelets (white arrow) and red blood cell (arrow head). Non-active platelets are small size with smooth surface.
Figure 2: (middle) Semi-active platelets are with one or 2 processes (white or black arrows) and increase their size.
Figure 3: (right) Active platelets are with 3 or more processes on surface (white or black arrows) and their surface becomes irregular or granular.

Criteria Used to Analyze Platelet Count Images

- Count total number of platelets on image, each sample count 4~ 6 images
- Semi-activated platelet: with 1 or 2 pseudopods
- Full-activated platelet: 3 or more pseudopods or conjugated platelets which groups of platelets that have pseudopods connected
- Non-activated platelet: small size and smooth surface without pseudopods or processes



		Baseline			24 hours			4 days		
		semi-active	active	Total plt count	semi-active	active	Total plt count	semi-active	active	Total plt count
Control n=8	Plt count	99.00	0.00	1203.00	217.00	13.00	2424.00	247.00	13.00	1279.00
	%	8.23	0.00	91.77	8.95	0.54	90.57	19.31	1.02	79.67
Oxygent n=8	Plt count	114.00	91.00	758.00	114.00	48.00	1445.00	102.00	15.00	1058.00
	%	15.04	2.51	82.45	7.89	3.32	88.79	9.64	1.42	88.94
Hespan n=8	Plt count	130.00	4.00	1320.00	145.00	4.00	2005.00	182.00	11.00	1877.00
	%	9.85	0.30	89.85	7.23	0.20	92.57	9.70	0.59	89.72

Figure 4. Percentage of active platelets and semi-active platelets. Cont =control group (n=8); PFC = Oxygent group (n=8); Hes = Hespan group (n=8). Plt = platelet. Platelets were count and calculated how many platelets were active or semi-active (see left table)

Table showed that the total platelets count for each group and semi / full active count as well as percentage of total count.

JMP Statistic Analysis: There are no significant differences in semi or full activated platelet count among groups

Analysis of Variance: Semi-active platelets

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Groups	2	110.5710	55.2855	0.8081	0.4591
Error	21	1436.6286	68.4109		
C. Total	23	1547.1996			

Means and Std Deviations (%): Semi-active platelets

Level	Number	Mean	Std Dev	Lower 95%	Upper 95%
Control	8	9.7975	4.87636	5.7208	13.874
Hespan	8	12.6250	9.65136	4.5563	20.694
Oxygent	8	15.0500	9.39708	7.1938	22.906

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Groups	2	104.3187	52.1594	0.7565	0.4817
Error	21	1447.9681	68.9509		
C. Total	23	1552.2868			

Level	Number	Mean	Std Dev	Lower 95%	Upper 95%
Control	8	6.1263	4.8357	2.0835	10.169
Hespan	8	11.0488	11.0378	1.8209	20.277
Oxygent	8	7.4100	7.8509	0.8465	13.973

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Groups	2	386.8232	193.412	1.6294	0.2199
Error	21	2492.7986	118.705		
C. Total	23	2879.6218			

Level	Number	Mean	Std Dev	Lower 95%	Upper 95%
Control	8	16.6763	15.9121	3.3734	29.979
Hespan	8	8.3363	5.2781	3.9237	12.749
Oxygent	8	7.9938	8.6638	0.7506	15.237

Analysis of Variance: full-active platelets

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Groups	2	15.30676	7.65338	1.0058	0.3827
Error	21	159.78624	7.60887		
C. Total	23	175.09300			

Means and Std Deviations (%): full-active platelets

Level	Number	Mean	Std Dev	Lower 95%	Upper 95%
Control	8	0.00000	0.00000	0.000	0.0000
Hespan	8	0.49875	0.93130	-0.280	1.2773
Oxygent	8	1.88750	4.68607	-2.030	5.8052

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Groups	2	175.4440	87.7220	0.9358	0.4080
Error	21	1968.5981	93.7428		
C. Total	23	2144.0422			

Level	Number	Mean	Std Dev	Lower 95%	Upper 95%
Control	8	0.81500	2.2255	-1.046	2.676
Hespan	8	0.09250	0.2616	-0.126	0.311
Oxygent	8	6.15500	16.6195	-7.739	20.049

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Groups	2	13.19803	6.59902	1.2089	0.3185
Error	21	114.62926	5.45854		
C. Total	23	127.82730			

Level	Number	Mean	Std Dev	Lower 95%	Upper 95%
Control	8	0.45125	0.53996	-0.0002	0.9027
Hespan	8	0.67375	1.27260	-0.390	1.7377
Oxygent	8	2.12375	3.80323	-1.056	5.3033

Platelet Rich Plasma (PRP) preparation:

1. 4.5 ml whole blood in blue tube was centrifuge 165g x 20 minutes.
2. Take all supernatant into 10 ml test tube, mixed with 0.1% glutaraldehyde in in 0.1 M cacodylate buffer into supernatant till 10 ml.
3. Centrifuge with 1200g x 15 minutes, remove all supernatant, keep solid residue
4. Add 2.5% glutaraldehyde in 0.1 M cacodylate buffer and re-suspend till 10 ml volume.
5. Process for SEM (in VCU imagine center)
6. Scope: ZEISS EVO 50XVP, Carl Zeiss SMT, Inc., Peabody, MA.

PFC was seen to stay in circulating blood at least for 24 hours

Conclusion:

- After intravenous infusion PFC, there is no morphologically significant change in platelet number and function.
- Qualitative and quantitative observation of platelet, changes in semi-activate platelets have shown a bigger variable than active ones.
- Current results are corresponded with the changes of coagulatory factor measurements.
- Therefore, intravenous infusion with Oxygent will not cause massive or severe coagulopathy.

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- **The work is funded by U.S. Army Medical Research and Materiel Command (W81XWH-13-1-0017 PI: Dr. Bruce Spiess)**

Effect of Intravenous Perfluorocarbon on Platelet Number and Function in Hemorrhagic Sheep

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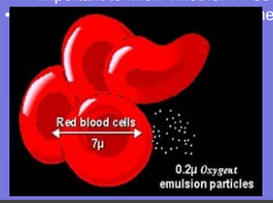
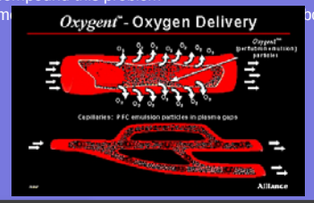
Introduction

- PFC is a non-polar oil/emulsion with enhanced respiratory gas (O_2, N_2, CO_2) solubility found in 1966.
- PFC Particles are smaller than RBC and can carry oxygen around blood clots and into tissues where blood flow is restricted
- PFC emulsion increases brain tissue oxygen consumption in traumatic brain injury investigations
- It increased arterial oxygen concentration in a decompression sickness model
- PFC was shown in a baboon model to cause thrombocytopenia (30-50%), warranting further investigation
- Previous study showed no significant PFC effects on platelets count and activation following a topload without shock.
- PFC has high potential for use in hemorrhagic shock situations, where platelet may already be affected. It is important to know whether PFCs would compound this problem
- …e phenom… …olic risks





Materials & Methods

Subjects and Groups:

- The experimental protocol was reviewed and approved by the Animal Care and Use Committee of Virginia Commonwealth University
- Total of 27 Juvenile Dorset or Dormer sheep (18-32 kg body weight) were used and randomly divided into 3 groups: PFC hemorrhage group (n=9); Saline hemorrhage group (n=9); and Sham surgery group (n=9)

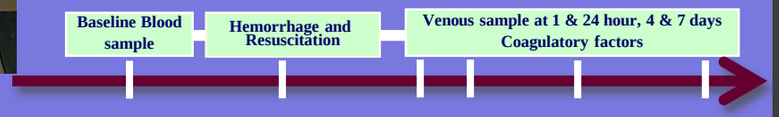
Hemorrhage Procedure & Blood Sample Collection:

- Animal was induced in anesthesia with 5% isoflurane and maintained with 2% isoflurane.
- Femoral cutdown performed bilaterally to draw blood, insert Swan-Ganz, and resuscitate as well as monitoring all physiological parameters: MAP, CVP, PA , CO, SVO2 etc.
- Sham surgery involved all of the above steps but not the hemorrhage or resuscitation.
- Sheep in hemorrhage groups had blood drawn in stepwise fashion until MAP was below 30 mmHg, maintained at that level (shock) for 60 minutes.
- Hespan resuscitation administered until sheep MAP maintained at 60 mmHg for 10 minutes.
- Either PFC (Oxygent, 60% v/w, 3g/kg or 5 ml/kg) or saline administered (5 ml/kg)
- Animal was allowed to recover from anesthesia and carried back to DAR facility after resuscitation completed.
- Venous blood was collected via jugular puncture immediately after resuscitation, 24 hours, and 4 & 7 days post trial

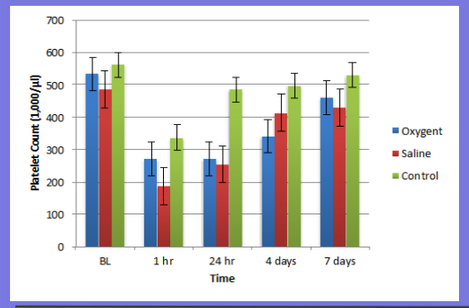
Blood sample measurement & Data analysis:

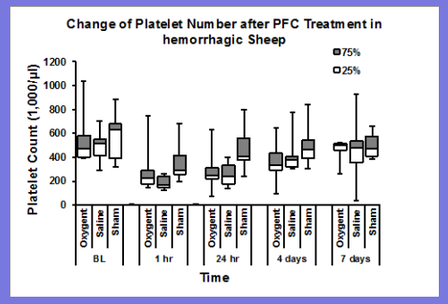
- Venous samples were measured for coagulatory factors including: platelet count, fibrinogen, Clot Elastic Modulus (CEM), and Platelet Contractile Force (PCF)
- Venous samples were collected at baseline, 1 hour, 24 hours, 96 hours, and 7 days after resuscitation

Experimental timeline



Results

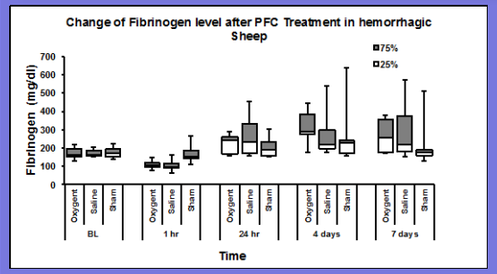




Platelet Number: In normal sheep, platelet mean value is about 400,000/μl. Our data showed that platelet count was significantly decreased at 24 hour after PFC or saline resuscitation compared with the sham procedure group or with their baseline. But there was no significant change in platelet number between PFC and saline groups at 24 hours post resuscitation. There was no significant difference in platelet count at 4 days and 7 days post shock among the groups. Platelet count returned back to baseline level at 7 days after shock..

Left Figure: Platelet count and mean distribution with standard error

Right figure: Box plot shows platelet count distribution with minimum value, maximum value, 25%, median, and 75% values

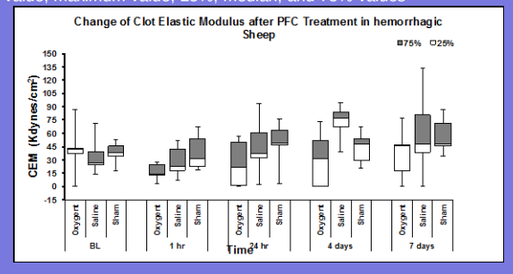


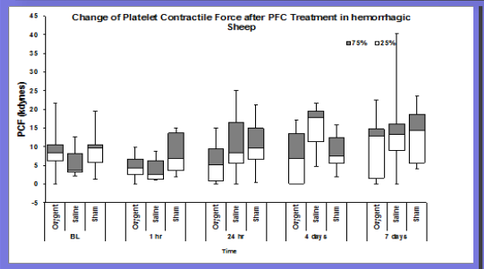
Fibrinogen measurement: there is no significant difference in fibrinogen counts between the 3 groups. There is also no significant change within each group, indicating no massive clot formation.

Clot elastic modulus: there is no significant difference in fibrinogen counts between the 3 groups

CEM measures platelet function, changing with production of force by platelets, rate of thrombin generation, and platelet concentration.

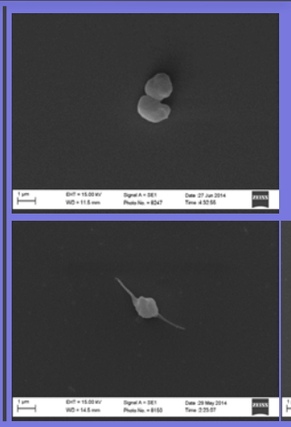
PFC does not over activate or significantly reduce platelet function based on CEM measurements.



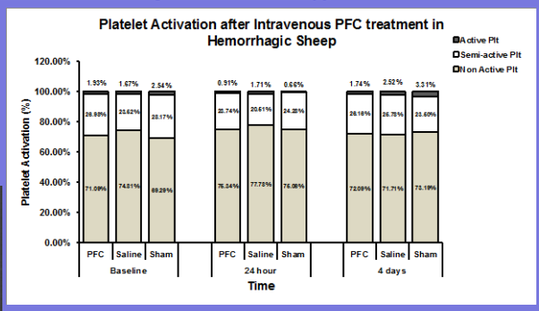


Platelet Contractile Force: there is no significant difference in fibrinogen counts between the 3 groups.
PCF measures platelet function and is affected by platelet metabolic status, GP IIb/IIIa status, and presence of antithrombin activity.
PFC does not over activate or significantly reduce platelet function based on PCF measurements.

Morphology of platelet observation with scanning electron microscopy



Top: non-activated platelets
Bottom left: semi-activated platelet
Bottom right: fully-activated platelets
Figure at right: There are no significant differences in platelet morphology between groups.



Criteria used to analyze platelet count images

- Semi activated platelet: 1 or 2 pseudopods
- Fully activated platelet: 3 or more pseudopods or conjugated platelets which are groups of platelets with pseudopods connected

Conclusion:

- After intravenous infusion PFC following shock, there is no significant change in platelet number, morphology, or function.
- In a sheep model, PFC does not cause significant effect on platelets

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- **VCU School of Medicine Summer Research Fellowship (Mentors: Drs. Bruce Spiess & Jiepei Zhu)**
- **The work is funded by U.S. Army Medical Research and Materiel Command (W81XWH-13-1-0017 PI: Dr. Bruce Spiess)**

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